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CONTENTS

NUMBER 1, FEBRUARY, 1934

Narcolepsy. LUMAN E. DANIELS........ 1

NUMBER 2, MAY, 1934

The Vitamins and Resistance to Infection. ELIZABETH CHANT ROBERTSON........ 123
The Potential Energies of Oxidation-Reduction Systems and Their Biochemical Significance. W. MANSFIELD CLARK........ 207

NUMBER 3, SEPTEMBER, 1934

Emphysema. W. B. KOUNTZ AND H. L. ALEXANDER........ 251
Observations on the Etiology and Treatment of Anemia Associated with Hookworm Infestation in Puerto Rico. C. P. RHOADS, W. B. CASTLE, G. C. PAYNE AND H. A. LAWSON........ 317

NUMBER 4, DECEMBER, 1934

The Etiology of Leprosy. EARL B. MCKINLEY........ 376

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NARCOLEPSY[1]

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Renewed interest in the subject of narcolepsy followed the discovery that its symptoms could be relieved by administration of ephedrine sulphate. More than 100 patients suffering from the malady have been examined at The Mayo Clinic since Cave submitted his thesis based on a study of the records of forty-two cases. The literature on the subject has accumulated so rapidly in recent years that an adequate review will soon be impossible. It seemed advisable, therefore, to attempt a careful study of all available material. My data are based on a review of the literature on narcolepsy, a study of the records of 147 cases observed at The Mayo Clinic, and my own experience in the examination and treatment of thirty-five of the 147 patients. I also interviewed twelve of the remaining 112 patients. Data relative to fifteen other cases were obtained through the courtesy of the attending physicians, with the exception of three in which the data were supplied by the patients themselves or their relatives. In all of the fifteen cases, the symptoms described were so typically those of narcolepsy as to leave little doubt about the diagnosis. The information given in many of the cases reported in the literature was so scanty as to impair its value for statistical purposes; the same objection applies to a lesser degree in several of the cases observed at the clinic. Some of the symptoms, consequently, may be more common than my results would indicate.

I was guided in the selection of material by Wilson's dictum that in the present state of knowledge, lines should not be drawn too sharply. The question of whether a person subject to attacks of sleep, but not to cataplexy, is suffering from "true narcolepsy" is more or less im-material. Too much valuable material is available in cases of this sort to permit their exclusion, much as it would simplify discussion. The arrangement of data proved difficult. The cases of "symptomatic" narcolepsy were often so similar clinically to those of "idiopathic" narcolepsy, and the relative significance of the different etiologic factors was often so difficult to evaluate, that rigid separation of the two groups seemed illogical. As the work progressed I came to doubt that there was such a thing as essential narcolepsy, and the final plan was to begin with a general consideration of etiology, symptoms, and course of the malady, and then to take up the question of so-called symptomatic narcolepsy.

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[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Neurology, March, 1932.

With the exception of articles published prior to 1910 in periodicals not easily accessible, the literature has been fairly well covered. The appearance of Redlich's (198) final contribution, when this study was in progress, was of considerable aid in the assembling of additional material.

DEVELOPMENT OF KNOWLEDGE OF THE DISEASE

Westphal, who in 1877 published the first good description of a case of narcolepsy, was reluctant to dismiss the peculiar attacks with the term "epileptoid" ("die Eigenartigkeit dur Zufälle—bleibt darum doch bestehen"). Fischer (60) reported a rather typical case the following year. Gélineau, whose report was published in 1880, believed he was dealing with a distinct entity, "un nevrose rare ou du moins peu connu," for which he proposed the term "narcolepsy." This term was used rather indiscriminately in the following years, many investigators being inclined to believe that in most cases so designated the patients were either hysterical or epileptic. Although Gélineau published a striking description of another case in 1894, the significance of cataplexy as a symptom was first recognized by Loewenfeld in 1902. Friedmann (65) caused considerable confusion in 1906 by using the term narcolepsy in reference to a condition now generally known as "pycnolepsy." Camp's (26) contribution, published in 1907, was the first description of the complete narcoleptic syndrome to appear in American literature.

The modern era of the study of narcolepsy opened with the publication of Redlich's first report in 1915, following which many other reports appeared in the German literature. Redlich had observed four cases of narcolepsy by 1916, and eleven by 1925; at the time he wrote the paper, which was published after his death, he had observed altogether nineteen cases. Among the contributions of German neurologists, Rosenthal's contains the most comprehensive consideration of the nature of the disease. Thiele and Bernhardt have recently completed a thorough study of twenty-five cases, but only a preliminary report of their work is available.[2] Among the French contributors, Lhermitte, in collaboration with Tournay, in 1927, presented before the International Neurologic Reunion a complete review on normal and pathologic sleep. In England Adie's contribution, in 1926, and Wilson's in 1928, did much to bring narcolepsy to attention. Adie, in 1930, published a concise account of his experiences with fifty cases which he had observed since the writing of his first paper. Interest in the subject has not been lacking in Russia, the papers of Wenderowič, Ratner, and Mankowsky being particularly worthy. Among the more recent American works are Spiller's report of four cases, published in 1926, and a more extensive contribution by Levin, published in 1929. Cave's series of forty-two cases observed at The Mayo Clinic was the largest group ever presented in detail. His paper appeared in 1931.

Janota and Skala, in discussing a paper read by Pelnar before a meeting of the Neurological Society of Prague, June 21, 1930, made a preliminary report on the successful treatment of narcolepsy with ephedrine sulphate. Their work was unknown to Doyle and me, not only at the time we made a preliminary report of similar experiences in October of the same year, but several months later when proofs of a later report were corrected.

SYMPTOMS

Attacks of sleep. Diurnal naps, coming on in response to a more or less irresistible desire to sleep, generally constitute, by reason of their frequency and persistence, the most troublesome manifestation of narcolepsy. The patient may be assailed soon after a good night's rest by drowsiness of an intensity normally experienced only by those who have been awake twenty-four hours or longer. Aside from a few rare cases (47, 77), the desire to sleep generally recurs daily or several times daily. Gélineau's first patient had as many as 200 attacks in a day, but some of these were probably cataplectic. Occasionally the patient may be free of the attacks for a few days every week, as in one of the postencephalitic cases reported by Stiefler, or they may disappear entirely for a few months, particularly during cold weather. At times the desire to sleep returns at such definite intervals that the patient, being forewarned, may more or less successfully conceal his infirmity. Many, however, can hardly afford to relax their vigilance for a second, for, like Chavigny's patient, who was "bien connu par ses accés de sommeil," they are constantly falling asleep.

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[2] The complete work appeared in print shortly before the final draft of this paper was corrected. Thiele, Rudolf and Bernhardt, Hermann: Beiträge zur Kenntnis der Narkolepsie. Abhandl. a.d. Neurol., Psychiat., Psychol. u. i. Grenzgeb. 69: 1–187, 1933.

Although patients with narcolepsy are usually fresher early in the morning than during the remainder of the day, some become very drowsy shortly after arising from a good night's rest; an unusually long night's sleep may even increase the diurnal drowsiness (219). As a rule, the attacks of sleep increase in frequency and intensity until early afternoon, which is usually the worst time of day for a narcoleptic patient. Many commence to brighten a little toward evening, and some are fairly wide awake by the time most persons are ready to retire. Most of my patients complained bitterly, nevertheless, of their inability to enjoy the evening's diversions. Drowsiness increased during warm weather in at least eight of the cases observed at the clinic, and in one reported by Münzer (155). In another case, stormy weather had an unfavorable effect similar to that noted by Gélineau and Mendel. Another patient became worse, and I know of two others who were no better, while living in high altitudes, although one of Collins' patients was better during a summer spent in the mountains.

Circumstances normally conducive to sleep are particularly trying for narcoleptic patients. Many of them are unable to remain awake after a heavy meal, during attendance at a lecture or recital, or when engaged in a monotonous piece of work. Interesting diversions, involving some excitement, providing they do not induce cataplectic phenomena, or, as in Gélineau's case, attacks of sleep, help to keep the patients awake. Outdoor exercise, when it does not prove conducive to rapid fatigue, may serve to dispel the drowsiness for the time being. Almost all patients fall asleep, at least now and then, on very inopportune occasions. The presence of strangers may not deter them from slumbering at table or during conversation. A few patients, including one seen at the clinic, have fallen asleep during coitus. Levin expressed the opinion that attacks under such circumstances were more likely to be cataplectic. Morton's patient, a physician, had fallen asleep when making a vaginal examination. If seized while on their feet, these patients generally do not fall, as a slight giving way of the knees may suffice to arouse them, or they may have time to sit down or lean against some support. A few continue to walk until awakened by a misstep or collision with some object. Since they can generally collect their wits, or at least recover their equilibrium when the occasion demands, they meet with serious accidents less often than might be expected. Pollock mentioned a brakeman who fell asleep without falling, while walking on the top of a train. The sleep-inducing properties of the monotonous hum of a motor render automobile driving particularly hazardous. Ordinarily, however, these patients have time to stop the car before they lose control, and although several have had accidents, I know of none who has been killed or badly injured under such circumstances. One of my patients wakened just in time to prevent himself from falling off a scaffold; another, overcome while cutting ice on a lake, fell into the water.

Although the attacks of sleep are assumed to be characteristically abrupt in onset (30), in some cases they seem to consist largely of a gradual increase of the patient's habitual drowsiness. They may come on without warning, as in one of my cases, and in the cases of Strauss (235), and of Paskind, or the patient may succumb in spite of his belief that he can ward off the attack. Generally, the patient is at least aware of a sudden and imperious desire for sleep. The preliminary sleepy feeling may sometimes be accompanied by a feeling of extreme physical exhaustion. Besides the sudden and overwhelming character of the drowsiness, various sensations and other experiences such as diplopia or hypnagogic hallucinations may enhance the character of the phenomenon, such as burning pain in the eyes, accompanied at times by failure of vision, pain in the head or neck, vertigo, paresthesia of the scalp, of a feeling of emptiness in the cranium. A generalized numbness or tingling, or a feeling of chilliness may accompany the onset.

One of my patients felt a tingling in her fingers; another experienced stiffness and drawing in the entire right side of the body. A child complained of hunger immediately before his attacks of sleep. Fröderberg's patient, whose attacks came on suddenly, and who felt at the time as though he could have "slept on the edge of a razor," saw a "flaming broom" as he passed into slumber. If the patient is able to keep himself awake, the various unpleasant sensations may increase to such a degree that he ultimately resigns himself to the inevitable; the depth and duration of the ensuing attack may be in direct proportion to the extent of his resistance. In one case, the sleepiness and all associated symptoms sometimes passed away if the patient persisted in his efforts, but this is unusual.

The depth of sleep in these attacks varies considerably in different cases, and at different times in the same case. Probably more often than not, the subject continues to be aware to some extent of what is going on about him. He may even join in a conversation, but the irrelevant character of his remarks generally betrays his failure to follow the subject. Certain narcoleptic patients continue to carry on some form of automatic activity when asleep. One of Goiffon's patients, a telegraph operator, while asleep and dreaming, could transmit correctly a line read before she passed into that state. Lhermitte and Roques (132) reported the case of a typist who produced legible although irrelevant copy during an attack; usually, under such circumstances, it is entirely illegible. The patient, when in a state of partial sleep with his eyes open, may "actually perceive but not fully comprehend the events of his environment" (202). The attack of sleep, on the other hand, may be quite sound and accompanied by dreams which, unlike the nocturnal dreams of a narcoleptic patient, are usually not unpleasant. One of Thiele's patients, for instance, dreamed that he had hypnotized his enemy into a helpless cataplectic state. The content of the dreams may enter into the patient's remarks as he awakens, much to the consternation or amusement of his companions. Two of my patients had experienced seminal emissions during their daily naps. Some narcoleptic patients start or jerk convulsively during the attack as might anyone in a state of troubled sleep.

The degree of muscular relaxation must be slight when the upright position is maintained, but in states of deep sleep it may be very marked, as I noted in two cases. Sometimes muscular relaxation sets in before the patient is asleep.

Occasionally the soft palate is relaxed to such a degree as to embarrass respirations. One of my patients, falling asleep suddenly a few hours after an operation performed under spinal anesthesia, breathed noisily and became cyanotic. He was aroused without difficulty and before falling asleep again he informed the resident surgeon that he had had similar difficulties with his breathing during previous sleeping attacks. He remained drowsy and cyanotic for several hours. A short, fat neck and an abnormally long soft palate may have been contributing factors. This patient had been subject to attacks of sleep on all occasions for the preceding three years. Aside from moderate hypertension and the anomaly just mentioned, nothing in particular was noted on general examination, although the patient died later of cardiac decompensation. A fellow countryman of the foregoing patient, but much more obese, snored loudly during his attacks and "almost choked" at times in the night. In Caton's case, spasmodic closure of the glottis during the diurnal naps as well as at night caused temporary cessation of respiration and marked cyanosis. Although the respiratory difficulties were evidently alarming to observers, they do not seem to have disturbed Caton's patient any more than they did mine. Morison's patient, an obese man, became cyanotic during his attacks of sleep. None of these four patients was subject to cataplexy.

Narcoleptic patients often respond when asleep to the slightest touch or the calling of their names. Any break in the monotony of stimuli coming from without may suffice to arouse them. Although responsive to external stimuli, and more or less conscious of being asleep, the patient may be entirely unable to shake off the somnolence by any effort of his own (151). At times, however, it may be impossible to arouse the patient or at least to keep him awake. This may be true only of the first part of the attack (85), during the early afternoon nap, or of the longer attacks (243). It was difficult at times to arouse one of my patients, and the patients reported by Benedek and Thurzó, Kahler, and Hilpert. Children suffering from narcolepsy, as might be expected, often resist being awakened. One child observed in the clinic during an attack of sleep did not respond at all to ordinary stimuli; when blood was withdrawn from his arm during an attack, he merely aroused momentarily to cry out and attempt to thrust his tormenters aside. Another young patient similarly fought off all disturbers. An older patient recalled that as a child she had kicked and screamed on being awakened from sleep. Since another patient, whose attacks appeared later in life, was inclined to scream when disturbed, this sort of behavior would not seem to be entirely confined to younger patients.

A narcoleptic patient resents being disturbed when he feels the need for repose as would a normal person under similar circumstances. If awakened before his needs have been gratified, he is often mentally dull and irritable. The patient whose cases Gélineau reported in 1894, remarked that when he became drowsy, sleep seemed the greatest blessing imaginable ("une bonheur extréme"); all social advances annoyed him greatly at such times. This patient recalled an acquaintance, similarly afflicted, who became furious if aroused from his naps. Noack's patient fell into a fit of rage on being awakened. At least nine of the patients seen in the clinic displayed more than ordinary pugnacity on being awakened; five of these, all adults, had struck those nearest and dearest to them, as had Hilpert's patient. One of the five behaved in this manner only if disturbed just as he was falling asleep. Although glad to have been awakened, and thoroughly ashamed of their conduct once they had collected their senses, they all seemed to have been governed for a moment by a feeling of uncontrollable anger. This behavior suggests an instinctive reaction to interferences with the gratification of an elemental urge; it serves to indicate the intensity of the desire of these unfortunate persons to sleep.

The duration of sleep depends largely on the circumstances under which the patient falls asleep. If, resigning himself to the inevitable, he seeks a quiet spot and assumes a comfortable posture, sleep may persist for from fifteen minutes to several hours. One of my patients would sleep until the following morning if he retired late in the afternoon, whereas if he remained up until later, nocturnal sleep was almost certain to be disturbed. If the patient is intent on something, the nap is likely to be momentary. One patient, falling asleep as the clock began to strike, awakened in time to hear the last stroke (30). One of Edel's patients, being overcome as he struck a match, awakened in time to light his pipe. Some patients awaken from a momentary nap feeling quite refreshed. In three cases observed in the clinic, the short naps were more refreshing than was the nocturnal sleep. Others feel more weary and out of sorts generally on awakening than they did before the nap, and continue to feel badly until after they have moved around for some time. Some patients feel hopeless and depressed on awakening; others experience a violent beating of the heart and are startled. Profuse sweating may supervene at the end of the attack.

I have been able to confirm the experience of others that the various features of these attacks of sleep are practically identical with those observed during normal sleep. At the onset of the attack, the patient's eyes become heavy and dull and his pupils smaller as his head inclines. He may attempt to raise his head, gazing rather stupidly at the observer meanwhile, but after a few vain efforts his eyes close and he has every appearance of being sound asleep. The pulse becomes slower than in the waking state, and the respiration more regular. In Chavigny's case the pulse rate was 40 to 50 beats each minute during the attack. Although the respiratory rate may become slower, 6 to 7 each minute, as reported by Poulalion and Meunier, this seems unusual. In a case of uncertain nature reported by Günther the patient's breathing during an attack of sleep presented features of the Biot type as well as of the Cheyne-Stokes type. Although the pupillary and tendon reflexes may be abolished and a Babinski reflex obtainable in normal sleep (110, 133), it is difficult to demonstrate these phenomena during the narcoleptic attack because any attempt at testing generally suffices to arouse the patient. Ordinarily, the narrowed pupils dilate to some extent as the lids are raised, and promptly react when light is flashed in. This effect of raising the lids may account for the dilated pupils observed by Bolten, and Kahler. In Noack's case, a former observer reported that the pupils failed to react during an attack. One of my patient's, a child aged seven years, who habitually fell into a deep sleep, was examined during an attack by two of my colleagues. The narrowly contracted pupils did not react to light, and none of the deep reflexes could be elicited. The plantar response during the attack was definitely that of extension. Since similar findings were obtained during a cataplectic attack in one of Wilson's (261) cases, their demonstration during an attack of sleep is of more than ordinary interest. On awakening from sleep, the patient may appear embarrassed or a little surprised, but he is almost always immediately conscious of having been asleep.

Attacks of sleep could be induced by hyperventilation in one of Strauss' cases, and in both of Serejski and Frumkin's cases. Redlich (198) had never succeeded in inducing an attack in this manner, nor had St. Lesniowski and Sznajderman in the case which they reported. As Redlich remarked, hyperventilation normally causes the patient to feel drowsy.

Cataplexy. The term refers to the state of helplessness into which a narcoleptic patient may be precipitated by emotional stress; he is not unconscious, but is a mass of toneless muscle, and he promtly recovers, none the worse for his experience. A normal person's jaw may drop slightly on embarrassment or surprise, or his muscles may become a trifle weak on hearty laughter (175), but few will agree with Curschmann and Prange that this manifestation of narcolepsy is not specific. The phenomenon would seem at any rate to represent the grossest exaggeration of what normally may occur during emotional states. The term cataplexy, first used by Adie (1) to designate this type of attack, has the sanction of general usage in the medical literature of Great Britain, France and America. It is defined in the Oxford dictionary, as "a temporary paralysis or hypnotic state in animals when shamming death" ("cataplessa" (G.) to strike down with fear or the like), which would be comparable, in some ways, to the cataplectic state observed in man. Redlich's term "affectiver Tonusverlust" has been used extensively in the German literature, but it applies to one feature of the attack only, leaving out of consideration the powerlessness of the victim (1, 2). Similar objections might be raised to Trömner's "affectatonie." Other terms which might be mentioned, are Henneberg's "cataplectic inhibition," Rosenthal's "affective adynamia," Stern's "tonus blockade," and Weech's "emotional asthenia."

Somer, who was apparently the first to publish a description, based on personal observation, of an attack of cataplexy, saw both of his patients while they were in that state. The second patient appeared to be a very tired patient with myasthenia. A neurologist in The Mayo Clinic, after seeing a patient in an attack of cataplexy, made the following note: "He looked like a patient with myasthenia gravis for thirty seconds, then, normal." Mankowsky also noted a similar resemblance.

Although the patient's face may become pale during the attacks, as noted by Somer, Wilson (261), and Mankowsky, and in one of my cases, no change of color was noticed by either Martin or Wohlfahrt. In one of my cases the face was flushed and the lips were blue. Mankowsky's patient perspired at the time. Few detailed observations have been made during these seizures because deliberate efforts to induce an attack do not often meet with success, and if an attack does occur, it is likely to be almost over before the examiner has recovered from his surprise. The pupils were observed by Somer, Goldflam, Strauss, Wilson, Ratner, and Serejski and Frumkin to be small during the attack; a similar observation was made in three cases observed in the clinic. The pupils were dilated in Martin's case and somewhat dilated in Mankowsky's. The pupils failed to react to light during the seizure in cases reported by Strauss, Ratner, Serejski and Frumkin, and Martin. The reaction was sluggish in Wilson's case, but apparently normal in the cases observed by Quensel, Wohlfahrt, and Mankowsky. Adie found that the reaction was "less brisk." In Ratner's case the pupils become dilated after the attack and remained so for several minutes in spite of the presence of bright sunlight. In one of my cases the pupils became a trifle larger as the patient recovered, and reacted sluggishly to light for a few minutes thereafter. Pupillary reactivity quickly returned, along with normal muscular tonus, in Martin's case. The tendon reflexes could not be elicited during the attack in two cases observed at the clinic, nor in Wilson's, Mankowsky's or Martin's cases. Adie stated: "The tendon reflexes are lost as soon as it becomes evident that an attack is occurring, and return as soon as recovery begins." The patellar reflex was sluggish during the seizure in Ratner's case, and immediately after recovery in Serejski and Frumkin's case. Fröderberg, on the other hand, found the deep reflexes unchanged. Idiomuscular excitability was lost, and the electric reaction apparently was reduced during the attack in Mankowsky's case. An extensor response to plantar stimulation was obtained by Wilson, but Adie had never seen anything suggestive of this phenomenon, although he had known the flexor response to become less marked after the onset of cataplexy. Mankowsky found the pulse rate a trifle increased during the attack, and Adie found it slowed.

Although laughter is the most common precipitant of cataplexy, being the only effective one in some instances, it is entirely without effect in certain cases in which anger or excitement, for instance, may regularly induce cataplectic phenomena. That the feeling of amusement may be equally as effective as the act of laughing, is attested by the utter inability of some patients to laugh even when highly amused; this was noted in Jakobsohn, and Levin's cases and in six cases at the clinic. However, the seizures became more severe in Jakobsohn's case when the patient's ability to laugh was restored after the onset of multiple sclerosis. The spasmodic laughing induced by the disease proved doubly annoying to this particular patient. A hearty laugh, furthermore, may be peculiarly effective, although the excessive mirth along might account for the intensity of the response. The beneficial effect of suppressing laughter or cultivating an artificial laugh could be attributed to the diverting influence of a voluntary effort which in itself sometimes helps to terminate a cataplectic attack. The element of surprise often enhances the effect of any given emotion. One of my patients, whose attacks were infrequent, had an attack only when something amusing occurred unexpectedly. The slight elation experienced on meeting an acquiantance, on receiving interesting news (235), or on coming on a familiar topic in the newspaper (119) may induce mild phenomena of cataplexy. In Nesbitt's case, the patient's yearning for a dress seen in a shop window proved effective. Not only fear, but a feeling of revulsion caused by the sight of a snake, or of water running from the mouth of a chicken rescued from drowning, has precipitated attacks.

The difficulty experienced, by reason of the infirmity, in responding to a call for sudden action may interfere with participation in various sports. Sometimes the muscles principally involved in the act attempted are the ones most affected. The arm may drop helplessly to the side when an attempt is made to throw a ball, or the patient may be unable to raise his gun to shoot. In one of my cases, the patient's jaw dropped and his eyes blurred if he made an attempt at repartee, and if he essayed a hasty retreat after the playing of some prank, his legs refused to function. Another patient was troubled by stiffness of the tongue if she tried to speak when embarrassed. A patient's inability to laugh when amused is probably on the same order. Resisting the desire to sleep may precipitate an attack, and the patients may awake from terrifying dreams in a state of helplessness as was noted by Wenderowič, and by Wilson, and in six cases observed at the clinic. Besides cases in which the legs gave way frequently early in the course of the malady, in six cases observed at the clinic, the seizures occurred spontaneously as well as under emotional stress. In two others, emotional excitement did not appear to have much to do with the attacks, although one of the patients fell more often when working under considerable tension.

The frequency of these attacks may be directly proportional to that of the attacks of sleep. Excessive fatigue, or a heavy meal, may aggravate the tendency to cataplexy as well as to somnolence. In one of Stiefler's cases, cataplexy was pronounced only on the days when the patient was having attacks of sleep. Cataplectic attacks may occur often early in the course of the malady when the patient is in a state of marked hypersomnia. Although in general cataplexy is less persistent than somnolence, the patient may fall a victim to it so frequently that it becomes the more annoying of the two. Certain patients can never break themselves of the habit of participating emotionally on all occasions, but many of them soon learn to curb their feelings, and with them the cataplectic tendency. Some cultivate a reserve, or even withdraw more or less from their associates. Others become so depressed from contemplating the apparent hopeless nature of their malady that their sense of humor becomes dulled. The ability of some patients, who ordinarily are susceptibile to emotional stress, to rise above their infirmity at crucial moments is worthy of comment. Gillespie's patient was able to rescue a child from drowning, although he collapsed a day later on thinking about the incident. A patient whom I interviewed recently had not experienced difficulty in punishing those who would make sport of his infirmity.

The onset of the cataplectic attack, like that of the attacks of sleep, may be accompanied by diplopia or blurring of vision; symptoms of this sort preceded the main part of the seizure in at least eleven of the cases observed at the clinic. The vision of two of the patients blurred completely at times; one of them remarked that both kinds of attack started in the same manner. Similarly, various types of paresthesia involving the scalp, shoulders, or entire body (267), a sensation of chilliness or one of warmth, may precede or accompany the onset of weakness. A patient of Rosenthal described cramps of the extremities and a drawing sensation throughout his entire body. Fröderberg's patient and one of Wenderowič experienced head noises at the beginning of attacks.

The severity and extent of the attack itself varies from a state of absolute powerlessness involving the entire voluntary musculature to limited involvement of certain groups of muscles, or a momentary sensation of weakness experienced more or less throughout the body. A slight giving way of the knees is probably the most common example of the milder attack; sagging of the jaw, combined with an inclination of the head and some weakness of the muscles subserving speech, would seem to come next in the order of frequency. Closure of the eyes and protrusion of the tongue are common. Although some patients drop objects which they happen to be holding, others retain sufficient power in their arms to permit them to grasp a support and thus prevent a fall. The progress of even the more severe attacks generally is not so rapid but that the patient has time to seek a chair or at least to glide easily to the floor. Speech may become entirely inhibited or indistinct, although some patients are able to talk in a low voice. The more or less generalized muscular jerkings, which often appear as the patient sags, have been attributed by Fischer (59) and Adie to voluntary resistance, each feeble attempt at movement being followed by a further relaxation of the respective muscles. Fischer (59) compared this feature of the attack to a fixation nystagmus. One patient seen in the clinic, in describing a trance-like state similar to cataplexy, said that if he attempted to move, all his muscles, particularly those of the face, twitched. The patient's attempt to contract the sagging muscles of his face and jaw may cause a peculiar grimacing similar to that seen if one is about to weep (1, 2). Occasionally, as in Fischer's (59) case, however, the jerking may be violent and partake more of the nature of a true motor discharge. In one case observed at the clinic, respiration ceased for about eight seconds during the attack; in another, the patient became dyspneic at the time of the seizure, as did one whose case was reported by Redlich (195). In the severe type of attack, muscular relaxation may be extreme; in Jakobsohn's case the patient's spastic limbs were observed to become completely flaccid during an attack of spasmodic laughter. If the patient's shoulders are raised, his head may roll from side to side, governed only by the force of gravity. The helplessness, rather than the loss of tonus, is emphasized in some accounts of the attacks. The wife of Burr's patient remarked that she could lead her husband around or throw him down whenever he happened to be pleased. One of my patients made the significant remark that her feelings during the cataplectic state were like those experienced in a terrifying dream. A man, on seeing a snake, was fixed to the spot, unable to move; a mother was unable to prevent her child from falling.

Among the less common features of cataplexy is a localized rigidity of certain muscles. Stiffness of the tongue has been experienced occasionally (76). Pelnar, and Skala each reported a case in which the patient complained of stiffness about the mouth; in two cases observed at the clinic the jaws felt rigid during the attack. It seems possible that in such cases the patient may have confused inability to move certain parts with true rigidity. So far as I know, the occurrence of an orgasm during an attack of cataplexy has not been reported to date. One patient informed me that if he fell in a certain manner while in a cataplectic state, he would have a seminal emission. This had happened two or three times, he believed.

The cataplectic attack is often only momentary, and, in general, seldom lasts longer than a minute or two. The duration of fifteen minutes reported by Quensel is probably the longest on record. Sometimes the patient must first control his mirth before he can recover from the seizure (193), but this is not difficult usually, for at such times feelings of amusement are generally replaced by a sense of disgust. Some patients, like the one referred to by Gélineau as a "modern Anteus," recover their strength as soon as they strike the ground. A nudge from another person may be just as effective in aborting the cataplectic attack as it is in aborting the attack of sleep. One patient recovered as soon as he could open his mouth; another could terminate the attack by rendering his tongue and toes tense, but considerable effort was required to accomplish this; still another obtained relief by pressing his tongue against the roof of his mouth. Although the patient is often none the worse for the experience, he may feel rather tired immediately on recovery; a few have been exhausted after the attack, as noted by Ratner and Kennedy, and in one of my cases. Nesbitt's patient continued to have cataplectic seizures until she could sleep undisturbed for a few hours; she was not subject, apparently, to attacks of sleep.

Although patients usually remain conscious, two patients seen at the clinic were not entirely aware, while in the cataplectic state, of what was going on. Martin's patient did not recall all that had occurred during a seizure which she had while under observation. Gillespie's patient dreamed during some of his cataplectic attacks, although he remained more or less in touch with his environment. In nine of the cases observed at the clinic, and in fourteen reviewed from the literature, cataplexy was said to be followed, occasionally at least, by a short nap. Sometimes laughter or excitement is followed immediately by sleep, as noted in one case in the clinic, and in cases of Gélineau, Bolten, and Tsiminakis. Patients not subject, strictly speaking, to attacks of sleep may feel drowsy during a cataplectic attack, or feel the need for rest when it is over. Münzer's (156) patient felt tired after the attack; a patient of Hoff and Stengel had a feeling that he slept for a few minutes during the seizure. Nesbitt's patient has been referred to.

The two principal manifestations of narcolepsy, although different at first glance, have much in common. Although the attacks of sleep generally appear spontaneously, they occasionally, like cataplexy, come on in response to emotional excitement; cataplexy, on the other hand, may occur spontaneously. Both types of attack may be preceded by similar phenomena, and sleep may follow a cataplectic seizure. Conditions which aggravate the one tendency may have a similar effect on the other. Although the degree of muscular relaxation is not so marked in the attack of sleep as in the cataplectic seizure, this is not always true. I know of one patient who, on falling into a helpless cataplectic state, could be restored by a brisk nudge. The attacks of sleep and the peculiar seizures of powerlessness to be considered later can often be terminated in a similar manner. Redlich, Adie (1), Wilson (261) and Lhermitte all expressed the opinion that the attacks of sleep and cataplexy were closely related phenomena, being different manifestations of the same process. Rosenthal contrasted the sleeping attack in which the patient continued to walk, with a severe cataplectic seizure in which, although fully conscious, he was unable to move a muscle. In the former, the higher psychic functions were more or less in abeyance although the static and motor apparatus remained active, whereas in the latter the reverse was true. Each represented a variety of partial sleep, or, in Rosenthal's words, a particular state of dissociation. Contrasting the ordinary attack of sleep with a cataplectic seizure, Lhermitte and Roques (130) wrote: "In the first, sleep is complete, while in the second, the inhibition involves the corporeal functions only."

Prodromes. The occurrence of various disorders of sleep early in the lives of certain narcoleptic patients, and the occasional appearance of isolated cataplectic attacks or other narcoleptic phenomena several years before onset, are both of particular interest because they suggest some fundamental defect in the function of sleep. Some patients begin to complain of diurnal drowsiness several years before the tendency becomes irrresistible (134); others say that they always have been sleepy. Paskind's patient and one of mine were abnormally drowsy during infancy. In Fröderberg's case and in one I have learned of through correspondence, the patients, both of whom acquired their narcolepsy at about the age of thirty years, always had been poor sleepers and subject to terrifying dreams. One of these patients, like one seen in the clinic, had been a somnambulist for years. One of Levin's patients had walked in his sleep up to the age of ten years, and for a few years thereafter had complained of terrifying dreams; one patient of Serejski and Frumkin had been a somnambulist in childhood. One of my patients had been subject to night terrors at about the age of ten years, and another, who seemed constitutionally drowsy, had experienced hypnagogic hallucinations after retiring, when he was seven years old.

The patient who was subject to night terrors in childhood had a sudden attack of powerlessness while conversing with friends four years prior to the appearance of attacks of sleep. One each of Bostock's and Stransky's patients had isolated cataplectic attacks a few years before onset. A few months prior to the appearance of attacks of sleep and immediately after severe physical exertion, Morton's patient was for two weeks a victim of almost complete insomnia. In the night, at this period, he often passed into a peculiar dreamy state in which he was a prey to many fantastic thoughts.

Onset and course to the time of admission or medical consultation. Although the two major symptoms of narcolepsy generally appear at about the same time, twenty-six of the patients examined in the clinic had attacks of sleep for periods ranging from two months to sixteen years before becoming subject to cataplectic seizures. Nineteen cases were reviewed from the literature in which from two months to almost eleven years elapsed before the cataplectic tendency became manifest. This would tend to undermine the belief that a person suffering from attacks of sleep alone is not suffering from true narcolepsy.

In the cases observed at the clinic, the interval elapsing between the respective appearances of the somnolent and cataplectic tendencies was sixteen years in one case, almost ten years in another, seven years in two, and four to five years in five; the interval in the remaining sixteen cases was three years or less. In the two cases in which the longest intervals were recorded, the onset was rather insidious; both patients had been subject to attacks of sleep only during the evening at first. An interval of almost eleven years was reported by Sperling and Wimmer; one of seven years by Serejski and Frumkin, and one of six years by Gélineau (70), and by Strauss (236).

The onset of cataplexy preceded that of the attacks of sleep by periods ranging from six months to two years in nine cases observed at the clinic, and in ten reviewed from the literature. The shorter intervals and the smaller number of cases in this group are more or less in keeping with the small number of cases reported in which cataplexy has not been associated with the attacks of sleep.

Cataplexy existed independently of the attacks of sleep for two years in cases reported by Fulton and Bailey, Levin, and Pelnar. In Pelnar's case cataplexy was replaced at the end of two years by attacks of sleep. In two cases at the clinic, twenty-two months and two years respectively elapsed before the patients began to have attacks of sleep in addition to cataplectic seizures.

Reference will be made to cases in which hypnagogic cataplexy with or without hallucinations, transient diplopia or hypnagogic hallucinations frequently recurred over considerable periods prior to the appearance of attacks of sleep and cataplexy.

When a complete cataplectic seizure is the first indication of narcolepsy, as in Westphal's case, the onset may seem very severe. In some cases, however, the patient may experience gradually increasing drowsiness, and at a later date be unable to recall the time when the desire to sleep first became irresistible. Sometimes the patient falls into a state of hypersomnia soon after the onset. Fracassi's patient, whose cataplectic seizures quickly reached a high degree of severity, slept from ten to fourteen hours daily soon after his symptoms became manifest. A patient examined in the clinic said that for the first three months he slept twenty-two to twenty-three hours of twenty-four. Another patient slept most of the time during a similar period. Both the attacks of sleep and cataplexy became very frequent a few months after onset in another case. The cataplectic tendency was so severe at the time of onset in four cases observed at the clinic as to keep the patients off their feet. One patient belonging to this group will be mentioned under "Injuries to the head." This and another patient remained in bed for the first six weeks because their legs gave way whenever they attempted to stand. In a third case, the patient lay around the house for the first few weeks for a similar reason. The fourth patient of this group, unlike the others, had recovered from a attack of influenza a short time before his symptoms appeared. He was kept in bed under observation for nine months, mainly because of the weakness in his legs, which was apparently not so marked as in the other three cases. All four patients slept most of the time they were lying around.

When the symptoms become pronounced early in the course, the improvement which is almost certain to follow within a year or less may be more or less progressive. All patients who gave a history of acute onset were still greatly incapacitated, however, at the time of their admission to the clinic. Cave's statement that "the symptoms reach their maximum intensity soon after they start and remain at that level for many years" applies, no doubt, in many cases. The symptoms were increasing in severity, however, at the time of the patient's admission twenty years after onset in two cases; ten years after onset in two other cases, and five years after onset in still another case.

A narcoleptic patient may seek treatment at any stage of his disease. Thirty-six of the 147 patients examined in the clinic had been afflicted, at the time of their admission, for at least ten years, and nine patients for twenty years. Two patients had had symptoms for twenty-eight years and one patient for thirty-five years; all three were women. The longer average duration among women with this malady might be used in support of the argument that they are less likely to seek treatment than are men. So far as the cases reviewed from the literature are concerned, the duration was, in general, longer among patients who did not have cataplexy. Blodgett's patient had been subject to attacks of sleep for forty-three years, one of Samain's for forty-two years, and one of Ballet's for thirty-eight years. Of the patients seen in the clinic who were not troubled with cataplexy, one had complained of abnormal somnolence for twenty-five years, and another for twenty-one years. Cases in which cataplexy was not accompanied by attacks of sleep have been of relatively short duration with the exception of one reported by Münzer (156), in which cataplectic attacks were becoming more troublesome ten years after their first appearance.

Spontaneous attacks of powerlessness; hypnagogic cataplexy. Some narcoleptic patients describe states of powerlessness into which they pass when sitting or lying down, and in which, although entirely conscious, they are often unable for a few minutes to speak or to move a muscle. Although engaged in a terrific internal struggle to move, the patient's appearance may be that of a person sleeping (261). Some of them occasionally can abort the attack by wriggling out of their chair or by making a supreme effort to regain control of their muscles before powerlessness becomes absolute. Others summon help by emitting a grunting sound, since a shake or even a light touch generally suffices to arouse them. The attack may be preceded by a roaring in the head, a snapping or pounding in the neck, or a feeling of numbness gradually spreading to involve the entire body. It may also be accompanied by a sense of constriction in the throat or thorax, often associated with a fear of impending death.

Attacks of this type were mentioned in six cases reported in the literature; in one of Ratner's cases, they constituted the first manifestation of narcolepsy. Nesbitt's patient, who was not subject to attacks of sleep, had attacks of powerlessness after retiring only on days when she had had no cataplectic seizures.

An attack of powerlessness occurred as a prodromal symptom four years prior to the appearance of other narcoleptic phenomena in one of my cases. Similar attacks were described by nine other patients seen at the clinic. In one of the nine, the patient continued, over a period of three years, to have several of these seizures in rapid succession, almost every night after retiring, until they became less frequent with the onset of attacks of sleep and cataplexy. In three of the nine cases, the state of powerlessness passed over into one of sleep.

The type of seizure under consideration is doubtless nothing more than a variety of cataplexy. It seems to come on under conditions conducive to sleep, and may be followed immediately by sleep, which perhaps might follow more regularly were it not for the terror which the experience instills in the patient's mind. In most instances, at any rate, the term "hypnagogic cataplexy" would seem to be quite applicable. The state is similar to the cataplexy which may supervene when the desire for sleep is resisted. It may be conceived of as a state in which the psychic and motor functions are not inhibited simultaneously as in normal sleep.

Hypnagogic cataplexy with dreams and hallucinations. In some states of powerlessness similar to the foregoing the inhibition appears to extend to the higher psychic levels, although the patient not only remains more or less conscious of his surroundings, but assumes a more or less critical attitude toward the dreams and hallucinations which he experiences at such times. Although these attacks may supervene during the day, they appear more often at night after the patient has retired. While lying or sitting in a helpless state, the patient may merely see persons walking about the room and hear them talk, but not infrequently the dreams and hallucinations are distinctly unpleasant if not terrifying. The hallucinations often have reference to animals, particularly those of which the appearance usually excites revulsion. Occasionally the experiences have a sexual coloring. The state may go over into one of complete sleep, or it may be necessary for the patient to arouse himself before he can settle down for the night. One of Serejski and Frumkin's patients, on falling into a state of powerlessness about ten minutes after retiring, would see troups of women who seemed to swarm about his bed and try to choke him. After a severe struggle he would seem to vanquish his assailants and the attacks would then be over. In a review of Nemlicher's report of a case of narcolepsy, mention is made of attacks of hallucinatory excitement with motor inhibition. Rosenthal (204) has recently called attention to similar phenomena. Seven of the narcoleptic patients examined at the clinic experienced attacks of the type under consideration. Details will be given in regard to three of these. The attacks recurred frequently in one case over a period of three years preceding the onset of attacks of sleep. They came on at first only after the patient had retired, being especially likely to appear after he had eaten rather heartily, or when he was very tired. At the onset of the trance, as he called it, his legs and then his arms felt numb and heavy, the numbness finally extending to his face. After he became entirely powerless, a ghost-like figure seemed to appear before him and then gradually fade away. After the attack, which seemed to last four or five minutes, he would fall asleep to awaken feeling quite refreshed four or five hours later.

In one case, attacks of powerlessness, preceded by a sensation like an electric shock, usually came on at night, and the patient had a feeling while in the attack that a snake was biting him in his right side or a rat crawling out through his skin in that region. He might also see and feel a snake coil itself about his neck, or see a brightly colored parrot that called him names. By exerting some effort he could generally recover his strength and rouse himself from the attack before falling asleep. The following is an extract from a letter received from this patient several months after he left the clinic:

> "At the present time they (the hallucinations) take the form of a sensation, rarely visual or auditory. I believe they would commonly be called nightmares. In the beginning, and I rarely let them get beyond that stage, they are more like a pain or pressure in one part of the body or another, with, of course, the lack or apparent lack of muscular control. If allowed to progress there will be a sort of soothing sensation of an almost pleasant nature; allowing the attack to continue, it almost invariably produces sexual excitation, seemingly independent of the conscious mind. What would happen if I allowed an attack to continue further I do not know, as an undefinable fear causes me to resist or break the attack."

The patient whose history follows had a much greater variety of experiences of a similar type.

An intelligent and healthy appearing woman, aged nineteen years, came to The Mayo Clinic July 13, 1931, complaining of a more or less constant feeling of tension. At the age of thirteen years, her legs began to give way frequently. Because of this she remained on a couch most of the day for two or three weeks, sleeping the greater part of the time. After this, her legs gave way less often, but when amused, she found that not only was laughter impossible but that she seemed to become weak momentarily. The drowsiness continued. She soon began to experience a more or less constant feeling of tension both in her head and body. This feeling apparently resulted from the constant effort she exerted to keep awake when reading, and to subdue the cataplectic tendency during participation in various sports. Any severe exertion seemed to increase this feeling of tension, which, persisting after she had paused to rest, thoroughly exhausted, proved very distressing. After she made a voluntary effort to relax, however, all strength seemed to leave her body and she would then pass into a state of partial sleep. About a year after onset, she began to have sensations of a sexual nature frequently when in this state. When she experienced these sensations she would awaken at once, feeling very refreshed for the time being, whereas if they failed to appear, she would continue in a troubled sleep for half an hour or longer and still feel tired when she awakened. Some time later, the sensations began to be accompanied by dreams, the content of which was either sexual or merely pleasant in character. The patient always remained more or less dimly aware of her surroundings during these relaxing spells as she called them. Although she might have as many as three in one day, she had been free of them for a month at times. These spells often preceded a succession of attacks of powerlessness which came on immediately after she had retired at night, and during which she was a prey to many fantastic and very vivid dreams, although consciousness seemed to be fairly well retained. Forms appeared at the windows and entered the room; she often felt as though snakes, birds, and other creatures were moving about in her abdomen and coming out of her mouth. She frequently dreamed that operations were being performed on her, always experiencing sensations appropriate to the given procedure; she occasionally dreamed of giving birth to a child. She frequently had a sensation, while in this state, of something like a huge abscess swelling up within her abdomen. This abscess would eventually burst and the escaping fluid suffuse her entire body, but she usually tried to arouse herself before this happened. During the early years of her malady, all these experiences terrified her greatly, but she could be easily aroused by her mother, who came in response to her screams. As a rule she was unable to relax completely and fall asleep, until she had passed through several of these states, and on awakening in the morning she might suffer a repetition of the same thing. About a year or two after the onset, the dreams ceased to be accompanied by states of powerlessness. After the patient began to take ephedrine sulphate, her various symptoms were almost completely relieved. Seven months after leaving the clinic she wrote that so long as she took the ephedrine regularly, her narcoleptic symptoms recurred only when she was overtired. She had been falling asleep in a normal manner every night and had experienced recurrence of her former relaxing spells on no more than six occasions. Without the use of ephedrine sulphate she was sure she would not be able to pursue her studies at the university.

When this patient first attempted to tell her story, it seemed so fantastic that she was considered hysterical, although her appearance and general behavior did not suggest that type of personality. The narcoleptic nature of her difficulties was first suspected when she mentioned the cataplectic phenomena. It was then learned through questioning that she was subject to attacks of sleep.

It must be emphasized that a state of powerlessness preceded, in most cases, the other phenomena of the attacks under consideration. The former could not, therefore, be attributed to the terrifying nature of the dreams. All patients were more or less aware of the unreality of their experiences, terrifying though they were at the time. One patient did not become convinced that it was all a dream, however, until he had looked about his room a few times after recovering from the attacks; another was glad to convince himself in a similar manner. Still another, on the other hand, said that her hallucinations did not trouble her because she always realized that she would soon fall asleep.

Attacks of cataplexy of awakening. A state of powerlessness similar to those previously considered may result from disturbance in the reversal of the inhibitory process of sleep, the psychic functions being released ahead of the motor functions. The patient will then awaken to find himself entirely incapable of movement, although fully conscious. The experience is usually terrifying, although the patient is often able, with great effort, to move some portion of his body and break the spell. This symptom is interesting because it may be encountered by persons not subject to the usual symptoms of narcolepsy. Rosenthal (202) was the first to report cases of narcolepsy in which the waking attacks, as he called them, had occurred. One of two brothers, whose cases he reported, had experienced a few attacks of this type, although he was not subject to cataplexy in the usual sense. This case has been included among the group of cases reviewed in which the symptoms were considered suggestive of cataplexy. Rosenthal referred to a similar association of symptoms reported by Friedmann (66), but in the latter's case the attacks of sleep were apparently not persistent. Another of Rosenthal's patients, a woman suffering from postencephalitic narcolepsy, had waking attacks while pregnant. Stransky's patient, one patient of Redlich (198), and four of the twenty-five patients observed by Thiele and Bernhardt had experienced this sort of attack. Burr, and Wahl's patients were, at times, unable to move for a few minutes after awakening from attacks of sleep. One of Bonhoeffer's patients had more prolonged attacks of this type. Lhermitte and Tournay used the term cataplexy of awakening in referring to these attacks as narcoleptic equivalents.

Only one of the narcoleptic patients seen in the clinic was subject, as far as I know, to waking attacks, unless the cataplectic seizures precipitated by terrifying dreams are included in this category. The patient had experienced some difficulty in arousing himself in the morning ever since the onset of narcolepsy eighteen months before. At intervals of a few days to two weeks during the preceding year, he had awakened to find himself in a powerless state. When the attack was pronounced, efforts to move were of no avail, nor did a shaking administered by his roommate help to terminate the attack. On the contrary, an attempt to awaken him from sleep had precipitated an attack of powerlessness. The patient would fall asleep again in a few minutes, and after a short nap awaken in full possession of his motive power. This manifestation of his narcolepsy proved to be the most distressing of all. While in the attack, he felt as though his mind was awake and active in a dead and paralyzed body. On other occasions his mind seemed foggy and his muscles weak when he first awakened, and it was only with considerable effort that he could arouse himself. He believed he had awakened in various other states having certain features of the two described, in varying degrees.

Waking attacks with dreams and hallucinations. Brock's patient was occasionally unable to move for from five to fifteen minutes after he had awakened from an attack of sleep. At times, while in this state, he would see various members of his family and hear them speak; at other times, he might feel the presence of a male friend who seemed to be exerting an evil hypnotic influence on him. Before recovering entirely, he would recognize the hallucinatory character of his experience and call to someone to arouse him. A patient seen in the clinic would occasionally awaken at night, thinking someone was in his room, but on attempting to move, he would find himself unable to do so for a few minutes. In one case, the process of awakening was often disturbed by a repetition of the attacks regularly experienced by the patient before she could fall asleep.

Transitional states. Many of the narcoleptic states in which the patient, although to all appearances sound asleep, is in reality aware of all that is going on about him, are no doubt, as Wilson (262) suggested, cataplectic. Although some patients, like one patient of Solomon's, merely have no inclination while in these states to participate in the events of their environment, others are quite helpless at the time. Certain related states in which the patient is less in touch with his surroundings than he may think he is, are more or less transitional, since they present certain elements of both the cataplectic and sleeping attacks. Besides attacks in which the cataplectic state was succeeded by an attack of more or less complete sleep, others have already been described in which the mental faculties were evidently inhibited to some extent at about the time, or soon after, the patient passed into a state of powerlessness. In attempting to separate attacks of sleep from those of cataplexy for purposes of consideration (the only good reason for so doing), it is very difficult to decide, in some instances, how a given attack should be classified. How, for instance, should the following description be interpreted? "When this sleepiness comes on, it does not feel exactly like one feels from loss of sleep; it just makes me feel helpless, but if I relax for a few minutes, it will leave me." Another patient, after describing an attack of spontaneous cataplexy, added that he was subject to a variety of seizures, representing all transitions from this state to one consisting only of transient clouding of thought, associated with an indefinite sensation about the throat.

The description of some of these transitional states of brief duration may suggest petit mal or pyknolepsy; in the absence of other attacks more clearly narcoleptic, a definite diagnosis might be impossible. I have, for example, never been able to decide with any degree of assurance whether the attacks to which Klieneberger's patient was subject were narcoleptic or pyknoleptic.

The conclusion that the attacks of sleep and cataplexy are manifestations of the same underlying process is, I believe, unavoidable. Since the opinion, shared by Rosenthal and Lhermitte, that cataplexy represents a state of partial sleep is supported by a variety of clinical observations, it should at least be regarded as a good working hypothesis. The peculiar susceptibility of the narcoleptic patient to emotional stress, however, remains unexplained. Since cataplexy is somewhat similar to the helplessness experienced in a terrifying dream, it may be that under normal conditions the reaction occurs only during sleep. The suggested analogy is, of course, purely speculative.

Hypnagogic hallucinations. Such hallucinations may be experienced more or less independently of the major narcoleptic attacks. If the patient is partially asleep at the time, he is generally not aware of the fact. Moyer's patient, for instance, saw strangely formed animals approaching him on several occasions in the course of a few months preceding the onset of his attacks of sleep. Fischer's (59) patient was subject to a variety of hallucinations during the day as well as after retiring. He could feel the fur and hear the chirping noise made by the animals that seemed to be scampering over his body. Like a patient previously mentioned he was always bitten in the same region by these imaginary creatures. Occasionally the experience was so real that he acted in accordance with it. Forms had appeared before one of the narcoleptic patients seen in the clinic when he was driving his car.

The association of two or more varieties of sensations seems characteristic of the phenomenon; the patient feels and hears, or feels and sees the various animals that appear in his dream. According to Lhermitte and Tournay's review of the subject of sleep, the visual and auditory phenomena of dreams represent an elaboration of sensations coming from within the patient's body. One of my patients, in fact, offered the suggestion that the hissing of the snake which he heard was really nothing more than the hissing in his own ears which regularly accompanied his attacks of powerlessness. Similarly, visions of rats running over the body may be prompted by the paresthesia experienced at the onset of attacks of sleep and cataplexy. The sense of being choked during a state of powerlessness may owe its origin to the dyspnea, and that of struggling with someone to the patient's attempt to regain control of his own muscles. When Serejski and Frumkin's patient had vanquished his imagined assailants, the spell was broken.

Amnesic states. A striking example of partial inhibition, or, as Rosenthal would say, dissociation, of the psychic functions is furnished by the peculiar fits of amnesia (or diurnal somnambulism) to which narcoleptic patients may be subject. My interest in these amnesic states was first aroused through hearing of a narcoleptic patient who would drive into a town where he had no intention of going, and be compelled to ask where he was; he would not have the slightest notion of how he got there. The following is an extract of a letter subsequently received from this patient:

> "At first I feel a little sleepy but not enough to stop to sleep; that is, I don't realize that I am as sleepy as I am, and the next thing I know I seem to brighten up and realize that I am miles beyond where I want to go, and have been driving while asleep, asleep with my eyes open. I don't remember anything about where I have been, and when I first wake up I don't have the slightest idea where I am or what I am there for. I usually turn around and go back until I find some familiar landmark along the road before I know where I am. When this sickness came on me, I was traveling on the road for a surgical instrument house. At first I went to sleep and wrecked my car several times before I finally gave up the position, but I have been driving a car while asleep for the last year and have driven many miles that way. I keep on my own side of the road and seem to have no trouble. When I wake up or come to, I feel rested as if I have had a good sleep with my eyes shut."

One of Levin's patients had no recollection, on one occasion, of a trip which she had just completed. One of my patients was lost frequently when driving on familiar streets. The following is an extract of a letter received from a patient who had been a narcoleptic for forty years:

> "I will be driving on a road that I have been over thousands of times, of which I know every tree and shrub when all of a sudden I am lost, I have no more idea where I am than if I had been dropped in China. This only

lasts, at the most, two or three minutes, and then the familiarity returns and I can place myself. There are two places where it occurs more frequently than at others. I call them my blind spots and if I have not had my accustomed amount of sleep, I begin to coach myself ahead of time. Turn right at Alms Hotel, one block, then left, over and over. By the time I am past this block I am all right again. The other place is just outside my own back gate and I have lived here for more than forty years!"

Another of my patients became amnesic on one occasion when delivering telephone directories. Suddenly realizing that he could not recall having traversed the preceding block, he retraced his steps to find that he had neglected to call at no more than three houses. Still another patient, while in a similar state, drove a cultivator to the corner of a field, alighted and set fire to some weeds that had accumulated on the implement. On coming to himself a few minutes later, he had no recollection of having started the fires or of having driven any further than the brow of a hill several rods from where he was standing.

Transient diplopia; transient ptosis; other ocular phenomena. The transient diplopia experienced when the desire to sleep is resisted is not peculiar to narcoleptic patients. Its occurrence independent of voluntary resistance at the onset of either an attack of sleep or cataplexy, however, would suggest that the mechanism subserving binocular vision is peculiarly sensitive to the inhibitory process. Like transient ptosis, fleeting diplopia occasionally occurs as an abortive narcoleptic attack, independent of other phenomena. Five narcoleptic patients seen in the clinic, apparently had double vision immediately before their attacks of sleep whether or not they resisted the desire; eleven had noticed diplopia, or blurring of vision, at the beginning of their cataplectic seizures. One patient, like Hilpert's, experienced diplopia on awakening; another, like one of Zehrer's patients, when reading. Four other patients had attacks of diplopia apart from their other symptoms, as did Silverstein's patient and a patient of Hoff and Stengel's. One each of Samain's, Spiller's, and Wilson's (262) patients had similar attacks about the time their other narcoleptic symptoms first appeared. Data relative to three patients, who experienced the symptom at about the time of onset of narcolepsy and shortly after recovery from an acute illness with symptoms suggesting encephalitis, will be found under "postencephalitic narcolepsy." Another narcoleptic patient seen at the clinic had noticed diplopia on two or three occasions shortly before the onset of narcolepsy.

Kennedy's patient began to complain of transient double vision three years before the appearance of other symptoms. The phenomena appeared about once a month at first, but gradually became of more frequent occurrence. Transient diplopia preceded the onset of narcolepsy by a similar interval in a case in which examination was made at the clinic; it was associated with severe headaches to which the patient was subject. The diplopia had been gradually becoming more frequent up to the time the patient was seen at the clinic, four years after the appearance of attacks of sleep and cataplexy. The patient had paresis, apparently congenital, of the right internal rectus muscle. His friends had observed the same eye turning outward during the attacks of diplopia. I have heard of another instance of early diplopia associated with headaches of a patient who had imbalance of the extra-ocular muscles. It seems entirely possible that narcolepsy might cause latent muscular imbalance to become manifest, for a narcoleptic patient would probably have more difficulty in maintaining fusion than would a normal person. One of Wohlfahrt's patients began to notice diplopia when laughing or when fatigued six months before onset. The diplopia later became permanent, but Wohlfahrt neglected to state whether the patient had paresis of any of the ocular muscles.

The transient diplopia occurring in cases of narcolepsy is not to be confused with the diplopia symptomatic of ocular palsy observed during the acute febrile stage of epidemic encephalitis. The diplopia of narcolepsy probably represents, as Janzen suggested, temporary failure of fusion.

In a paper on the dissociation of the components of sleep, Bonhoeffer mentioned a typical case of narcolepsy in which the patient had attacks consisting of sudden closure of the eyes and falling forward of the head. The head could be raised at once, but the eyes remained closed for a few minutes; occasionally the patient fell asleep before he could open the eyes. Bonhoeffer expressed the view that these attacks represented a localized cataplectic phenomenon, a view with which I am entirely in accord. One of my patients had noticed partial ptosis, more marked on one side than the other, on numerous occasions during the six months following the onset of his symptoms. A narcoleptic patient recently admitted to the clinic, informed me that some of his attacks consisted only of sudden closure of his eyes. Unlike Bonhoeffer's patient, however, he was able to open them at once.

One of Wilson's (262) patients experienced difficulty in averting his gaze at times. During these seizures he would find himself gazing at an object as in a day dream, the object finally becoming indistinct. The attack could be broken only with some effort, and on one occasion the immobility spread until it involved the entire body. A patient seen at the clinic was likewise unable to avert her gaze at times. Another was likely to fall asleep if she looked fixedly at an object.

Disturbed nocturnal sleep. In addition to the difficulties of getting to sleep at night, and of awakening in the morning, to which a narcoleptic person may be subject, the nocturnal sleep itself may be disturbed by insomnia and terrifying dreams. In fifty-two cases of the complete narcoleptic syndrome, with information relative to nocturnal sleep, reviewed from the literature, some degree of insomnia existed in thirty-two. Dreams were mentioned in nine of the thirty-two cases, as well as in eleven of the remaining twenty in which the patients were not wakeful. In all but nine of the fifty-two cases, therefore, sleep was disturbed in some manner. Of eleven patients subject to attacks of sleep only, six slept poorly at night; one of the six and two of the remaining five, who said they slept well, dreamed excessively. In a case reported by Münzer (156), of a patient subject to cataplexy only, the night's rest was broken by insomnia and bad dreams. The records of 119 of the cases of narcolepsy observed at the clinic contained information relative to nocturnal sleep. Of the 102 patients of this group who had cataplexy, sixty-four did not sleep well at night; thirty-eight of the sixty-four, and four of the remaining thirty-eight who slept well, had many dreams, chiefly unpleasant. Some disturbance of nocturnal sleep was mentioned, therefore, in sixty-eight of the 102 cases of narcolepsy with cataplexy. Seventeen cases, in which cataplectic attacks did not occur, remain. Insomnia was recorded in five cases of the latter group, and excessive dreaming in three others. One patient whose sleep was not otherwise disturbed, walked in his sleep, being similar in this respect to a patient of Curschmann and Prange.

Although narcoleptic patients who are troubled with insomnia do not become wakeful as a rule until two to four hours after they have fallen asleep, some never sleep soundly. Four patients who were seen in the clinic slept better in the day; one of these, a nurse, preferred night duty for this reason. Another patient who had not slept soundly for twenty years, made the significant remark that only parts of his brain slept. Some patients, on the other hand, sleep from ten to twelve hours at night.

The nocturnal dreams of narcoleptic patients are generally either terrifying, or at least distinctly unpleasant; some find this phase of the malady distressing. These dreams, like those experienced in the hypnagogic state, are, as a rule, extremely vivid. The patient dreams of fighting, often against overwhelming odds, of being unable to escape from assailants, man or beast, or of being in some other dangerous or distressing situation from which there is no escape. The helpless condition in which the patient often finds himself during the dreams may persist into the waking state; the result is an attack of cataplexy. One patient subject to experiences of this sort, said that his dreams had been bad enough ever since the beginning of the narcolepsy, but that about the time he first became subject to cataplexy, four years prior to his admission, they became "horrible." He dreamed of snakes and monsters, of being chased by wild animals, of lying on an infant and being unable to move. The constant sense of fatigue, as well as the futility of the patient's struggle against his infirmities may be symbolized in the dreams. One of my patients often dreamed of being compelled to read a newspaper continuously; the accompanying feeling was intense exhaustion. A good example of the fantastic is furnished by the case of a woman who dreamed on one occasion that countless numbers of ground moles were being pressed out of her abdomen; on another, that long chains of sausages were being withdrawn. She ascribed dreams of this character to her extreme obesity, concerning which she was very sensitive. On still another occasion this patient dreamed that cats were coming out of her mouth.

Since disturbances of nocturnal sleep are by no means a constant feature of narcoplesy, some doubt may be entertained in regard to a statement that they constitute one of the essential features of the malady. The disturbances are sufficiently frequent, nevertheless, to war-

TABLE 1

Weight gained at time of onset in cases of narcolepsy

SOURCE OF DATA	AGE AT ONSET, YEARS AND SEX	AMOUNT GAINED AND TIME INTERVAL
Caton	30M	56 lb. (25 kgm.) in 1 year*
K. Wilson	24M	56 lb. (25 kgm.) in 19 months
	16M	21 lb. (9.5 kgm.) in 2 months
Bonhoeffer	39M	24 lb. (11 kgm.) in 3 months
Skala	24M	22.5 lb. (10 kgm.) in 7 months
Correspondence	31M	55 lb. (22.7 kgm.) in 6 months
	35M	45 lb. (20 kgm.) in 2 months
	25M	20 lb. (9 kgm.) in 2 months
	15M	35 lb. (16 kgm.) in 4 months
	31F	30 lb. (13.6 kgm.) in 8 months
	20M	30 lb. (13.6 kgm.) in 2 years
Examination at The Mayo Clinic	9M	12 lb. (5.4 kgm.) in 1 month
	16M	26 lb. (11.7 kgm.) in 2 months†
	30M	20 lb. (9 kgm.) in 3 months†
	29M	20 lb. (9 kgm.) in 4 months†
	15M	67 lb. (30 kgm.) in 5 months
	23F	55 lb. (25 kgm.) in 5 months
	29F	45 lb. (20 kgm.) in 6 months
	24M	40–50 lb. (18–22.7 kgm.) in 6 months
	14M	40–50 lb. (18–22.7 kgm.) in 6 months
	22F	35 lb. (16 kgm.) in 6 months*
	9F	100 lb. (45.5 kgm.) in 1 year
	24M	30 lb. (13.6 kgm.) in 1 year
	14M	75 lb. (34 kgm.) in 2 years
	32F	100 lb. (45.5 kgm.) in 3 years (50 lb. first year)
	11F	58 lb. (21.8 kgm.) in 3 years*
	14F	44 lb. (20 kgm.) in 3 years
	29M	75 lb. (34 kgm.) in 4 to 5 years
	22M	55 lb. (25 kgm.) in 5 years
	24F	59 lb. (27 kgm.) in 6 years* (39 lb. first 2 years)

* No cataplexy.

† Not obese at the time of examination.

rant their inclusion, as Thiele and Bernhardt suggest, in all hypothetic considerations. The troubles of a narcoleptic patient are often not ended when he retires; he may fall asleep in an abnormal manner, his rest may be broken by terrifying dreams or prove unsatisfactory in other ways, and he may have further difficulties during the process of awakening. In short, both the nocturnal and diurnal sleep may be abnormal. In some instances the entire cycle of sleeping and waking is profoundly upset, although the complete reversal characteristic of epidemic encephalitis does not seem to have been observed in cases of narcolepsy. Fröderberg has suggested that the sleep-regulating mechanism of a narcoleptic patient is poorly balanced.

Gain in weight. Instances of such rapid and early gains in weight, as are recorded in table 1, although not common, have aroused considerable interest. In one of my cases numerous fresh striae cutis distensae furnished striking evidence of the rapidity with which subcutaneous fat had accumulated. A gain in weight was known to have occurred at the time of onset in twenty-four cases reviewed from the literature, including eleven of encephalitic origin. Seventy-three (almost half of the narcoleptic patients seen in the clinic) were known to have gained weight since the onset, this tendency being more marked among women.

Among the cases encountered at the clinic, seventeen of the seventy-eight men subject to attacks of sleep and cataplexy had become obese since the onset of narcolepsy; with one exception, they apparently began to gain about the time the symptoms first appeared. At least seven of the nine, previously obese, continued to gain weight after onset. Of eighteen other men, who were obese at the time of examination it was not recorded when the increase in weight was first noted, but since the excessively obese in this group all had had narcolepsy for many years, it seems quite likely that most of the eighteen had acquired their excess weight in the course of the malady. Four men, not overweight when examined, were certain that they had made a substantial gain in the first few months following onset. Thirteen of the thirty-eight women suffering from the two major symptoms of narcolepsy had probably become obese since onset. Eleven of these apparently had begun to gain at about the time the symptoms were first noticed; one of the eleven may have started to gain a short time in advance of the first signs of drowsiness, as did Skala's patient. Five of a group of eleven women who were previously overweight continued to gain after onset; one of these gained 100 pounds in three years. Four, who were not obese when they came to the clinic, had gained weight shortly after they became subject to attacks of sleep and cataplexy.

Only one of the nineteen men not subject to cataplexy had begun to gain weight when he first became subject to attacks of sleep. Another began to gain three years after onset, and at least five of the seven who were previously obese did not cease gaining with the advent of narcolepsy. Three of the twelve women who did not have cataplexy had become obese since the onset of narcolepsy.

In general, the most marked increases of weight seemed to occur in cases in which the onset of narcolepsy was rather abrupt, and the patient slept excessively from the beginning. The patient who gained 100 pounds in the first year said that she seemed to sleep most of the time during that period. Three patients who gained rapidly had abnormal appetites after the onset of the narcolepsy; two others whose gain was more gradual had very good appetites. In other cases, attempts at dieting were not very successful. Some patients curtailed their intake of food because they were less sleepy when eating lightly.

Of the patients seen at the clinic, only five had lost weight since the onset. Two of these, the only ones definitely under-weight, were not subject to cataplexy and presented unmistakable evidence of chronic encephalitis.

Abnormal growth. As the anabolic processes are assumed to take precedence over the catabolic during sleep, the early gain in weight observed in narcolepsy might be attributed, in part at least, to the hypersomnia itself. For similar reasons, somnolence might favor the progress of normal growth. In this respect, the frequent onset of narcolepsy during adolescence, when a certain amount of drowsiness would not be considered abnormal, may have some significance. Three narcoleptic patients seen in the clinic gave histories of rapid growth at the time of puberty or shortly after. One patient who was fairly large when he became subject, at the age of sixteen years, to narcoleptic attacks, continued to grow thereafter and gained weight rapidly. Another patient, aged fourteen years at onset, asserted that, following the appearance of symptoms, he gained 3 to 4 inches (about 7 to 10 cm.) in two months. In the following two years he had, as his father remarked, changed from an undersized boy into a well-built young man. The third patient grew rapidly and was rather sleepy during his thirteenth year, but did not become subject to attacks of irresistible sleep and cataplexy until he was twenty years old. Hilpert's patient also began to grow rapidly at about the time narcoleptic symptoms appeared during his fourteenth year.

A huge mulatto, aged thirty-five years, whose case was reported by Thrash and Massee, said that he seemed to keep right on growing and that he could never wear more than three or four pairs of shoes of the same size. A rather tall man seen at the clinic said that since the onset of narcolepsy at the age of twenty-nine years, his hands and feet had become larger and his features coarser. Since the examining physicians evidently did not find his appearance suggestive of acromegaly, the changes may have been attributable to accumulations of subcutaneous fat. Cases have been reported in which the patient's appearance was thought to be acromegaloid, but in none of these was a progressive change in the features described, with the exception of Gruszecka's case of postencephalitic narcolepsy, in which signs of acromegaly, which later regressed, were said to have developed in a man, aged twenty years.

The sexual function. Disturbances of normal sexual development, or impairment of the sexual function following the appearance of narcoleptic symptoms, although evident in only a few of the reported cases, are not without interest. Dercum's patient, who had become subject to attacks of sleep at the age of fifteen years, was impotent and had small testes. One of Levin's patients, a boy aged fifteen years, had small testes and a eunuchoid habitus, but pubic hair had appeared shortly before Levin saw him. In Möllenhoff's case, a youth aged seventeen years, potentia had not yet developed. A youth, aged nineteen years, whom I examined had never experienced any particular sexual desire, and could recall having had nocturnal emissions on only two occasions. In a case reported by Strauss (235), in which sexual maturity had been retarded, the usual symptoms of narcolepsy did not appear until the patient was twenty-five years of age. Redlich (195) reported two cases and Levin one case in which, although potentia developed at the proper time, libido was lacking, but Redlich (198) learned subsequently that one of his patients acquired more than the ordinary amount of desire as he grew older. Libido was also lacking in one of my cases, that of a married man aged twenty-one years. In a case reported by Stiefler, in which the onset occurred in early childhood, libido was poorly developed and potentia had become impaired shortly before Stiefler was consulted. Strauss, Redlich, and Rosenthal commented on the immature appearance of their patients.

In one of my cases, desire was reduced to a considerable degree after the appearance of symptoms when the patient was sixteen years of age. He attributed his deficiency to the overwhelming drowsiness which came over him when the day's work was done, as well as to the social embarrassment occasioned by his infirmity. The impotence, which was first noted nine years after onset in another case observed at the clinic may have been psychic. Both desire and potentia had been considerably reduced since onset in three other cases involving young men. Two other men, who had been indulging excessively, complained that they were deriving less pleasure from the act than formerly, and that their sexual powers were somewhat diminished. Among the postencephalitic cases that have been reported, hypolibido was noted in Gruszecka's case, and impotence in a case each of Lhermitte and Kyriaco. In the remaining cases, the symptoms of narcolepsy appeared in the third decade or later. Among Gélineau's patients, one suffered a loss of desire shortly after onset of narcolepsy, and another, sixteen years after the appearance of symptoms. Libido became impaired after the onset of symptoms in Jolly's, Fischer's (59), and Stern's (227) cases, and in one each of Zehrer's, and Serejski and Frumkin's cases, as well as two cases observed at the clinic.

Potentia had become reduced not long after onset of symptoms in one each of Wohlfahrt's, and Hoff and Stengel's cases; another of Wohlfahrt's patients had become completely impotent. Potentia had become impaired nearly twenty years after onset of the disease in a case observed at the clinic. In another case, libido and potentia had both decreased since the appearance of attacks of sleep three years before.

In summarizing the cases reviewed from the literature, it was found that libido was impared or absent in twelve cases, potentia in nine, and both libido and potentia in four, making a total of twenty-five cases in which some impairment of the sexual function existed. Of ninety-seven male narcoleptic patients seen in the clinic, desire alone seemed to have been affected in three cases, potentia was diminished or absent in five, and both libido and potentia were adversely affected in six. The cases in which sexual development was impaired have been included in this summary.

Little is known about the effect of narcolepsy on the sexual functions of women. One of Rosenthal's patients lost all desire following an attack of epidemic encephalitis, during the course of which she became subject to cataplectic seizures. One of the women seen in the clinic had also lost all desire since the onset of narcolepsy. The menstrual flow was scanty in cases observed by Pfanner, and Audo-Gianotti, in one case observed by Adie, as well as in six cases observed at the clinic. Two of the six patients had failed to menstruate for months at a time, although one had been twice pregnant. One of my patients, who in addition to an excessive amount of hair on her body had a masculine type of habitus, failed to menstruate until the age of seventeen years; her periods of flow remained scanty for several years. I have heard indirectly of another case in which puberty was delayed. Twenty-one of the women admitted to the clinic suffering from narcolepsy had been pregnant since the onset of their malady.

Polyuria and polydipsia. So far as I know, no case of narcolepsy associated with diabetes insipidus has been reported, although I have heard of a young girl who passed large amounts of urine. Both Pfanner's, and Henneberg's patients were said to have mild polyuria, although in Henneberg's case the volume of a twenty-four hour specimen amounted only to 2,400 cc. One of Redlich's patients had moderate polyuria and polydipsia. Three patients seen in the clinic complained of frequency of urination and increased thirst, but only one of these appeared to be passing an excessive amount of urine. Of seven other patients who, like Fulton and Bailey's patient, complained of increased thirst but not of frequent urination, one, whose polydipsia was mainly nocturnal, excreted 1,500 cc. of urine between 7 p.m. and 7 a.m. The volumes of specimens of urine submitted by the other six were well within normal limits. Two other patients excreted rather large amounts.

Headaches; epistaxis. Narcoleptic patients often have headaches immediately before attacks of sleep, particularly if the latter are resisted. At least twenty of those seen in the clinic became subject to frequently recurring headaches at the time the other symptoms appeared, or some time later. One patient had begun to complain of constant headache and associated scotoma a year after the onset. The headaches, to which another patient had been subject since childhood, became more severe after he began to have attacks of sleep and cataplexy. Epistaxis seems to be a precursor rather than a symptom of narcolepsy. In Fulton and Bailey's case, however, a creeping sensation about the right temple, and a feeling of fullness in the head, first noticed about a year before, were often relieved by epistaxis. One of my patients, who had hypertension, had had two bouts of epistaxis in the year preceding her admission.

Miscellaneous symptoms. Somnolence appeared to be aggravated by moderately severe constipation in three of the cases observed at the clinic. In thirty-two other cases the patients were constipated, twelve to a mild or moderately severe degree, and twenty to a slight degree. Twelve patients complained of abdominal distress, the symptoms being referable to disease of the biliary tract in three, and to duodenal ulcer in two others. The remaining seven patients complained for the most part of gaseous distention. Two patients, including one from the preceding group, complained of poor appetite; another complained of a constantly bad taste in his mouth. Two were troubled with vertigo; in one case this symptom was relieved, with the other difficulties, by treatment with ephedrine sulphate; the other patient evidently had vertigo of the so-called toxic type. Another patient, like one of Gruszecka's, had been subject to asthmatic attacks since childhood. Another had hay fever. One patient came to the clinic principally for relief of Raynaud's disease, the onset of which had preceded narcolepsy by eight years. One of Bolten's patients, a youth aged sixteen years, was subject to acrocyanosis in cold weather.

RESULTS OF EXAMINATION

General appearance; body weight. The typical narcoleptic patient is sleepy but well nourished and appears full-blooded. If not too plethoric, as were two patients seen in the clinic who died later, the appearance is indicative of good health. A slightly strained expression about the eyes, arising apparently from the constant effort to keep awake, is characteristic. Some of the patients relate their experiences in a rather sheepish manner, whereas the more self-contained maintain an air of mild boredom. The features of those who are so drowsy as to appear dull and stupid may sometimes lead to the mistaken diagnosis of parkinsonism.

Some degree of obesity was encountered in 56 per cent of the cases of narcolepsy seen at the clinic (table 2). The obese tendency was somewhat more marked among women than among men in cases in which attacks of sleep and cataplexy were both present. Four women of this group were, respectively, 96, 91, 81, and 77 per cent overweight. Among the patients not subject to cataplexy, on the other hand, the men had a greater tendency to obesity than did the women. The weights of two men of this group were, respectively, 70 and 71 per cent in excess of normal. One woman of the group, however, was 83 per cent overweight.

TABLE 2

Weights of 147 patients with narcolepsy examined at The Mayo Clinic

SEX	TOTAL CASES	UNDERWEIGHT, CASES	10 TO 24 PER CENT OVERWEIGHT, CASES	25 TO 50 PER CENT OVERWEIGHT, CASES	MORE THAN 50 PER CENT OVERWEIGHT, CASES	PERCENTAGE OVERWEIGHT NOT KNOWN, CASES	OVERWEIGHT, TOTAL CASES	WEIGHT IN TERMS OF PERCENTAGE OF STANDARD WEIGHT*
With cataplexy:								
Men	78		28	9	2	5	44 (56%)	114 (69 cases)
Women	38	1	8	8	4	2	22 (58%)	125 (32 cases)
Without cataplexy:								
Men	19	2	2	4	3	2	11 (58%)	121 (16 cases)
Women	12	3	3	2	1		6 (50%)	116 (10 cases)
Total	147	6	41	23	10	9	83 (56%)	119 (127 cases)

* Weight could not be determined in terms of percentage of standard weight in twenty cases because heights were not recorded.

In my experience, the distribution of fat in cases of narcolepsy is fairly uniform if the usual individual variations are taken into consideration. The young woman who was 83 per cent overweight, had, like Solomon's patient, a physique approaching the masculine. The build of one of the men in the series of patients seen at the clinic suggested, according to Cave, the adult Froelich type. This was true of one of my patients so far as the distribution of fat was concerned. Pollock made brief mention of a case in which attacks of sleep of fifteen years' standing were associated with a typical Froelich syndrome, although on examination no evidence of a tumor of the hypophysis or brain could be found. In a typical case of narcolepsy reported by St. Lesniowksi and Sznajderman, the obesity was said to be hypophyseal.

In Henneberg's, and Dercum's cases as well as in one of Redlich's and one observed at the clinic, acromegaly was commented on. It is doubtful, however, that much significance should be attached to isolated observations of this kind. The question of large stature of some narcoleptic patients will be considered later. In Pfanner's case the hands and feet of the patient were large, but the receding lower jaw, the dry and waxy skin, the fine and dry hair, the senile facies and the falsetto voice suggested hypopituitarism rather than hyperpituitarism, although the basal metabolic rate was a trifle high.

Eyes. Various types of nystagmus, usually of slight degree, slight anomalies of the pupils, and differences in the width of the palpebral fissures appeared to be no more common among patients with narcolepsy than among any other group of patients. Homonymous hemianoptic scotoma was noted in a case mentioned by Holmes; the visual fields in a case observed at the clinic revealed homonymous notching of the left upper quadrants.

Skin. The tendency to dermographia, often referred to in the literature, was present in practically every case of narcolepsy which I have observed. The skin is occasionally dry, although a tendency to sweating is not uncommon, and the integument seems to be normally moist in most cases. The skin was dry in Pfanner's case and in one each of the cases reported by Levin, Wohlfahrt, and Serejski and Frumkin. In Levin's and Wohlfahrt's cases, the basal metabolic rates were rather low. In a case observed at the clinic, that of a woman aged sixty years, who was subject for ten years to attacks of sleep but not to cataplexy, the skin was dry; the basal metabolic rate was −18. A physician has written to me in regard to a girl, aged fifteen years, who has had attacks of narcolepsy for more than two years, whose skin and hair were dry and who did not perspire. When her basal metabolic rate was raised from −24 to −10 by the administration of desiccated thyroid gland, the skin became normally moist. The dry, scaly skin and brittle hair, observed at the clinic in anòther case, was found to be a family characteristic. One patient had generalized vitiligo which had appeared two years prior to the onset of narcolepsy

Miscellaneous data. General examination of narcoleptic patients seen in the clinic revealed little that was abnormal. Two patients had considerable dental sepsis, and two others, marked tonsillar sepsis. A diagnosis of chronic sinusitis was made in still two other cases. The heart was moderately enlarged in one case, and evidence of disease of the mitral valve was found in another. A rather marked degree of prostatitis existed in three cases, complicated in one by gonorrheal arthritis. The presence of a pelvic tumor was demonstrated in two cases. Disease of the biliary tract was present in three cases and a duodenal ulcer in two other cases.

Temperature. Oral temperatures were recorded in 133 of the cases observed. at the clinic. As in Redlich's (198) cases, there was no constant deviation from the normal range; the frequency of occurrence and the extent of the variations were, in general, no greater than might normally be encountered in any other group. Two patients had slightly subnormal temperatures when in hospital. Considerable fluctuation of the temperature had been observed, apparently, by the patient's physician at home during the prolonged attacks of sleep in one case observed at the clinic. During the four days this patient was under observation, however, his temperature remained within normal range.

Pulse rate. The slow pulse characteristic of the attacks of sleep, has been observed in the waking intervals. Audo-Gianotti's patient had a pulse rate of 52 beats each minute and Pfanner's, a rate of 56. The pulse rate varied between 48 and 52 in one of Bolten's cases, and between 56 and 76 in a case of Strauss (235). A rate of 48 was recorded in one of Strauss' postencephalitic cases. Redlich found a normal rate in all of his cases. One of Edel's patients had slight bradycardia. The child whose case Maclagan reported, on the other hand, had a pulse rate of 140. The rate was below 60 beats each minute in six of the cases observed at the clinic. One patient, whose rate was 54 at the time of examination, said that his physician at home found it to be 43 on one occasion. In two other cases rates of 56 and 68 and a single rate of 56 were recorded. The three lowest rates observed in individual cases were, respectively, 47, 48 and 50. The pulse rate exceeded 90 beats each minute in thirteen cases, reaching 120 in two and 116 in one case. The average pulse rate in the entire group of 147 cases was 79 beats each minute.

Blood pressure. Essential hypertension was present in fourteen of the cases observed at the clinic. A mentally disturbed girl had a systolic blood pressure of 160 mm. of mercury, and a diastolic pressure of 110. The remaining thirteen patients were thirty-six years of age or older. A systolic pressure of less than 100 mm. of mercury was recorded only three times among the adults and adolescents; a reading of 86 mm. in one case being the only one less than 92 mm. Redlich found the systolic pressure to be a trifle low (90 to 100 mm. of mercury) in several of his cases and somewhat elevated in one case only.

LABORATORY EXAMINATIONS

Urine. Evidence has been presented to show that polyuria is rather uncommon in general in cases of narcolepsy. Twenty-four hour specimens of urine were collected in twenty-one of the cases observed at the clinic. The specimens exceeded 2,000 cc. in volume in two cases only. In one case the volumes of two successive twenty-four hour specimens were 2,480 and 2,900 cc.; the specific gravities were 1.007 and 1.012, respectively. In the other case, a single twenty-four hour specimen had a volume of 3,500 cc. and a specific gravity of 1.020. Twelve hour specimens were collected in eighty-nine cases. The volume of these specimens, which in many instances probably were not collected with any particular care, exceeded 1 liter in nine cases and 1,300 cc. in three. In the latter group one twelve hour specimen contained 2,000 cc. of urine, another, 1,650 cc., and a third, 1,500 cc. The specific gravity of the three specimens was 1.010, 1.006 and 1.014, respectively.

Repeated studies of the relations between intake and output of fluids, in Redlich's cases failed to reveal any constant deviations from the normal, although the output occasionally exceeded the intake. The effect of the administration of pituitary preparations on excretion of urine was found to be normal in five of his cases. In Münzer's postencephalitic case, the patient's capacity for diluting the urine was considered normal. The water balance apparently was not disturbed in Paskind's case. In three of my cases, carefully controlled studies of water balance were carried out over a period of three to four days, with normal results. A fourth patient kept a record of intake and output for three days, the average difference in favor of the intake being approximately 770 cc. As this patient was very active and perspired rather freely, the existence of actual retention of fluid seemed unlikely. The available information would seem to indicate that a disturbance of water metabolism is not an essential feature of narcolepsy.

Blood: hemoglobin, erythrocytes, lymphocytes, and eosinophils. The general impression that narcoleptic patients are full blooded would seem to be borne out by the relative rarity of anemia among the reported cases. The number of erythrocytes was said to be a trifle low in one of Redlich's cases; Guleke's patient was described as anemic. In Lhermitte and Peyre's (128) case, on the other hand, erythrocytes numbered 7,600,000 and 8,000,000 in each cubic millimeter of blood on different occasions, and the concentration of hemoglobin was 19 grams in each 100 cc. Erythrocytes numbered 6,800,000 in one of Somer's cases, but the concentration of hemoglobin was not given. In a case of a man subject to attacks of sleep only, reported by Pitres and Brandies, the values were as follows: hemoglobin 106 per cent (Gowers); erythrocytes 6,960,000 and leukocytes 10,300 in each cubic millimeter of blood. The blood of Jolly's patient was said to be thick and dark. The possible relation of narcolepsy to polycythemia will be considered later.

The concentration of hemoglobin was determined in 145 of the cases of narcolepsy observed at The Mayo Clinic; the erythrocytes were counted in 109 of these. Although the concentration was less than 80 per cent (Dare) or 14 grams in each 100 cc. (Sheard-Sanford method) in sixteen of the 145 cases, and the number of erythrocytes was a trifle low in two other cases, in only one case was the anemia at all marked. Values for hemoglobin greater than 80 per cent (Dare), or 17 grams for each 100 cc., were reported in thirty-one of 145 cases observed at the clinic. In five of the thirty-one cases, the erythrocyte counts were a trifle high, as they were in four other cases in which the concentration of hemoglobin was not elevated; the highest count was 5,800,000 and the next highest, 5,400,000. It must be concluded, therefore, that narcoleptic patients are more likely to be slightly plethoric than anemic, although in most cases the number of erythrocytes and the concentration of hemoglobin were within normal limits. Whereas all but one of the nine patients whose erythrocytes were a trifle high were overweight, this was true of only nine of the twenty-six patients the concentration of whose hemoglobin alone was in excess of normal. Only one of the sixteen patients who revealed some degree of anemia was underweight, eight being overweight. The concentration of hemoglobin bore no apparent relation, therefore, to the patient's state of nutrition.

Relative lymphocytosis was found to be common among the cases reported in the European literature. Of forty-two cases in which figures were given, the proportion of lymphocytes was in excess of 30 per cent in thirty-two and more than 40 per cent in seventeen. In four of Beyermann's cases, the percentages ranged from 50 to 54; one of Rosenthal's patients had lymphocytosis of 53 per cent. Lymphocytosis was found in seven of the nineteen cases seen by Redlich (34 to 45 per cent). One each of Levin's, and of Weech's patients had slight lymphocytosis, these being the only instances of this particular symptom in the American literature on narcolepsy. The forty-two cases in which the percentages of lymphocytes were given included seven postencephalitic cases. The only American case in this latter group (40) was also the only case in which the proportion of lymphocytes was not increased. In a case of Rosenthal, the lymphocytosis was slight, but in the remainder the percentages ranged from 40 to 44. There was slight lymphocytosis in Möllenhoff's case, and definite lymphocytosis was "almost constant" in Thiele and Bernhardt's series of twenty-five cases. In three other cases reported, the percentage of lymphocytes was apparently within the limits of normal.

A differential blood count was made in forty-four of the cases observed at The Mayo Clinic. The proportion of lymphocytes exceeded 30 per cent in seventeen cases and 35 per cent in five, but in only one case was the porportion as high as 40 per cent. In five cases it was less than 20 per cent. The average percentage of lymphocytes for the forty-four cases was 28.6. The leukocytes numbered less than 10,000 for each cubic millimeter in all of the seventeen cases in which there was lymphocytosis, with the exception of one case in which the count was 11,000. In only four of the entire group of forty-four cases did the number of leukocytes exceed 10,000, the highest count was 11,700. The lowest count reported was 5,000. The ages of the seventeen patients who had lymphocytosis ranged from twenty-one to fifty-eight years, the average age being approximately thirty-one years. The greater frequency of lymphocytosis among cases reported in the European literature is difficult to explain. Wilder recalled that, in 1919, when living conditions in Vienna were poor, the sleepiness of which he and others complained was associated with definite lymphocytosis. Lymphocytosis occurring in cases of narcolepsy is generally assumed to be indicative of underlying disturbance of the endocrine system, a view that is hardly in accord with the experience of hematologists (157).

Eosinophilia of 10 per cent occurred in one of Levin's cases, and of 7 per cent in Mankowsky's case following encephalitis. The proportion of eosinophils was in excess of 4 per cent in three of Redlich's nineteen cases; 6 was the highest percentage reported. One of the patients at the clinic had eosinophilia of 12 per cent, which could be accounted for by urticaria from which she was suffering at the time of her examination. In the remaining forty-three cases in which a differential count was made, the proportion of eosinophils was as high as 4 per cent in only two. Eosinophilia appears, therefore, to be of infrequent occurrence among narcoleptic subjects.

Serologic tests for syphilis. In one of the cases observed at the clinic, the Wassermann reaction of the blood was weakly positive on one occasion and negative on another; the patient was pregnant at the time. In another case, the reaction was strongly positive on two occasions, although the spinal fluid was negative. The serologic tests of the blood for syphilis gave negative results in the remainder of the cases in which examination was carried out at the clinic.

Cerebrospinal fluid. In thirty-nine of the cases reviewed from the literature, the cerebrospinal fluid was examined. In thirty-one of these, including eight postencephalitic cases, the fluid was negative. The pressure of the fluid was equal to 26 mm. of water in Lhermitte and Nicolas' (127) case; in one of the cases reported by Lhermitte and Roques (132) it was equal to 51 cm. of water; routine tests of these fluids yielded negative results. Both patients improved following withdrawal of a quantity of fluid. The fluid in one of Somer's cases contained 13 cells in a cubic millimeter, and a trace of globulin, the latter being absent in a sample of fluid removed subsequently. Although the patient was thought to have contracted syphilis many years before, the Wassermann reactions of blood and spinal fluid were negative. In one of Wagner's cases, the spinal fluid contained 5 cells, and in one of Levin's it contained 8 (examination under low power magnification). A trace of globulin was found in Silverstein's case and in one of Janzen's. The protein content of the fluid was thought to be slightly increased in one of Ratner's cases. The cerebrospinal fluid was examined in seven of the cases observed at the clinic, in all of these the Wassermann, Nonne and colloidal gold tests yielded negative results. The number of cells was within normal limits in every case, and in the five cases in which manometric readings were made the pressures were normal. Four other patients had had the cerebrospinal fluid examined prior to admission to the clinic, presumably with negative results.

Blood sugar. In the cases of narcolepsy reported by Fischer (59), Strauss (235), Weech, Brock, Willis (two cases), Levin (two cases), Wahl, and Grün, the concentration of sugar in the blood, as observed in a state of fasting, ranged from 75 to 105 mgm. for each 100 cc., the average for the group of eight cases being 89 mgm. The values were said to be normal in several of Redlich's cases. Thiele and Bernhardt found that in their cases the concentration of sugar "lay on the lower limit of normal." Considerable variation was encountered in the postencephalitic cases that have been reported. In the cases of Crouch, and Maclagan, for instance, the values were high, amounting to 150 and 184 mgm., respectively. The blood of Münzer's patient contained 128 mg. of sugar, but when the sugar tolerance was tested, the fasting value was found to be 112 mgm. In one of the postencephalitic cases reported by Tsiminakis, on the other hand, the value was a trifle low, that is, 72 mgm. The high concentration reported in one of Strauss' (236) cases was a misprint, the actual value being within the limits of normal.

The concentration of sugar in the blood as observed in a fasting state was recorded in twenty-one of the cases observed at the clinic; the values ranged from 66 to 140 mgm. for each 100 cc., the average for the group being 89 mg. The concentration of sugar exceeded 101 mgm. in only one case of this group. Low values were obtained in three cases. In the case of a boy who complained of hunger shortly before an attack of sleep, the fasting value was 66 mgm. Blood removed during an attack, several hours after the patient had eaten, contained 87 mgm. of sugar in each 100 cc. In another case the value was 70 mgm. In the last of the three cases, the fasting blood sugar was reported as 77 mgm., but when the sugar tolerance was tested, the fasting level was at 83 mgm. The patient's statement that he occasionally ate candy in order to keep awake was probably of little significance, since he was usually very drowsy after eating a heavy meal.

Sugar tolerance was normal in one of Solomon's cases, in two of Willis', and in three of Levin's, as well as in the single cases reported by Wilson (259), Maclachlan, Skala, and Paskind. In two other cases reported by Levin, the initial rise of the blood sugar following the ingestion of glucose was slight and, one hour later, the level had begun to fall. In one of Wohlfahrt's cases, and in Münzer's postencephalitic case, although the initial response was good, the sugar level began to fall early. Tests of tolerance to sugar in twelve cases of narcolepsy that were observed at The Mayo Clinic yielded normal results in six. In three cases, the blood sugar was below the fasting level two hours after the ingestion of 100 grams of glucose, and in another three hours after the glucose meal, the values ranging from 56 to 66 mgm. In two cases, the blood sugar was still appreciably above the fasting level at the end of three hours.

Wohlfahrt attributed to hyperinsulinism the delayed or slight response of the level of the blood sugar to subcutaneous injections of epinephrine in three of his cases. The blood sugar increased promptly and adequately, however, following injection of 1 mgm. of epinephrine in Münzer's case.

Blood calcium. Redlich found the concentration of calcium in the blood to be within normal limits in several of his cases. Weech, Wahl, and Fröderberg reported values of 9.9, 10 and 13 mgm. for each 100 cc., respectively. In seventeen of the cases observed at the clinic, the values ranged from 8.8 to 10.6 mgm., the average being 9.9 mgm.

Basal metabolism. The results of basal metabolic studies are summarized in table 3. Although the rates reported in cases reviewed from the literature show greater individual variation, the general trend is the same as in the cases observed at The Mayo Clinic. Cases in which there was no suggestion of cataplexy did not receive separate consideration in part "A" of the table because there were only three cases of that type, in which a rate was reported. Rates obtained from patients receiving medication with thyroid or from pregnant women were naturally disregarded. Otherwise, an average was made of all rates obtained in each case. In one of Levin's cases, for example, in which a rate of −34, the lowest on record for a case of narcolepsy, was obtained, the average of two rates was −30. The rates included in part "B" of table 3, with three exceptions, were determined in the basal metabolic laboratories of the clinic. Although an effort was always made to keep the patients awake during the test, a few informed me that they fell asleep in spite of the efforts of the laboratory personnel,

TABLE 3

Basal metabolism in cases of narcolepsy

SEX	CASES				RATES		
	Total	Below −10	Normal −10 to +10	Above +10	Lowest	Highest	Average
A. Rates in forty-two cases reported in literature							
Men	30	12	17	1	−30.0	+24	−6.4
Women	12	7	4	1	−26.4	+20	−11.3
Total	42	19	21	2	−30.0	+24	−7.8
B. Rates in seventy-three cases observed at the clinic							
With cataplexy:							
Men	35	9	26	3	−17	+14	−3.7
Women	21	15	5	1	−24	+16	−10.1
Without cataplexy:							
Men	5	2	1	2	−16	+16	−1.0
Women	12	7	4	1	−21	+12	−9.5
Total	73	33	36	7	−24	+16	−6.5

this may have tended to make some of the rates unduly low. There seemed to be no constant difference between the rates of obese patients and the rates of those who were not obese. Among the men subject to cataplexy, those who were obese had slightly lower rates, as a rule; among the women, on the other hand, the average rate of those whose weights were not excessive, was definitely lower.

Repeated determinations of the basal metabolic rate in a large series of cases would be very desirable. Information obtained from a fairly large series in which only single observations, for the most part, were made in individual cases, may permit a few tentative conclusions to be drawn. The figures assembled here indicate that although a marked individual variation in the metabolic rates is encountered, low rates occur more commonly than high rates in the general run of cases of narcolepsy. Although most women have rates of less than -10, more than half of the men have rates within the range of normal.

Roentgenographic examination of the sella turcica. The results of roentgenographic study of the sella turcica have been of considerable interest to physicians interested in narcolepsy. This is largely due to the attempt to correlate anomalies of that structure with phenomena attributable to dysfunction of the hypophysis and the vegetative centers in the tuber cinereum and other portions of the floor of the third ventricle.

In cases of narcolepsy reported in the literature, the sella turcica was described as "extraordinarily small" in six cases, as "small" in eight, and as "rather small" and a "trifle small" in two others. Anomalies of doubtful significance were noted in nine other cases. In forty-two cases, including nine of postencephalitic narcolepsy, the sella turcica was said to be normal; in nine others, including two postencephalitic cases, the skull was reported as normal on radiographic examination, without particular mention being made of the sella turcica. In twenty-five of a group of seventy-six cases, therefore, various anomalies were reported.

Roentgenograms of the skull were made in 108 cases of narcolepsy at The Mayo Clinic. In thirty-seven of these, the sella turcica was reported as normal. In sixty-two cases in which the skull was reported to be normal without particular mention being made of the sella turcica, it seems fair to assume that no gross variations of that structure existed.

A bridged sella turcica was reported in six (5.5 per cent) of the cases, which corresponds rather closely to Camp's (27) incidence of 6 per cent for this anomaly. The sella turcica was small in two cases. The anteroposterior diameters as measured by Camp were 0.8 cm. and 0.9 cm. and the depths 0.8 cm. and 0.6 cm., respectively. In both cases, the measurements were above the minimal normal length of 0.5 cm. and the minimal normal depth of 0.4 cm., as determined by Camp in his study of 500 roentgenograms of the head in which the sella turcica had been reported normal. The sella turcica was slightly eroded in one case, apparently as a result of increased intracranial pressure of long standing. Experience at the clinic would seem to indicate, therefore, that anomalies of the sella turcica are not abnormally common among patients with narcolepsy.

It is difficult to draw any conclusions in regard to reports in the literature that the sella turcica is small, since the criteria used in individual cases are not known. The assumption that a small sella turcica is indicative of a small hypophysis seems hardly justified (192, 199), nor does there appear to be any evidence to support Ratner's suggestion that anomalies of the sella turcica are indicative of a constitutionally inferior diencephalon.

Encephalography. Normal encephalograms were obtained by Thiele and Bernhardt in seven cases and by Wagner in one case. Two of the narcoleptic patients, examined at The Mayo Clinic, submitted to encephalography. In one case, the results were negative, in the other the encephalograms were thought to be suggestive of cortical atrophy, although there was no clinical evidence of mental deterioration.

EFFECT OF NARCOLEPSY ON THE PERSONALITY

Patients with narcolepsy are usually sensitive about their infirmity. Irresistible drowsiness may come over them in situations demanding the utmost dignity; the cataplectic tendency not only exposes them to the mortification of collapsing in a more or less helpless state in the presence of strangers, but may also reveal emotions they wish very much to dissimulate. Deceived by the appearance of normal health and strength which these patients usually present, friends and relatives often feel that they are lazy or indifferent; strangers may take them for drunkards or drug addicts. Even the air of reserve and forced gravity which they assume to prevent cataplectic seizures may be misinterpreted. Some of the patients, particularly those who look on their infirmity as a personal weakness, see some justification in the reproaches of their associates. It is not surprising, consequently, that narcoleptic patients frequently become shy and depressed and avoid social intercourse. Most of them, however, continue in their efforts to earn a livelihood.

Samain commented on the tendency of narcolepsy to attack its victims at a time of life when its effects were especially likely to be demoralizing, that is, during adolescence and early adult life. One of the younger patients seen at the clinic did not seem desirous of obtaining relief, but his attitude was unusual in this respect. At least five others had achieved some degree of success in college work in spite of the difficulty of remaining awake in the lecture room and during study hours.

Irritability was mentioned in fifteen cases reviewed from the literature, and in twenty-three of those observed at the clinic. The latter group included five of seven cases in which the patients had become nervous, and one of four cases in which some degree of emotional instability was recorded. All patients aged twelve years or less on admission had become irritable. Three presented definite behavior problems, although apparently the disturbance was not so profound as that likely to be observed in the postencephalitic state. In one of the three cases there has since been definite improvement in behavior, coincident with that of the major symptoms of narcolepsy. Partial control of the attacks of sleep by the administration of ephedrine in another case, however, was not accompanied by any improvement in behavior, which continued to be disturbed whenever the patient became fatigued. Levin's first patient, who had been good-natured previously, became irritable, argumentative and indolent. Anderson's patient became emotional and subject to outbursts of temper. Increased irritability and decreased tolerance to alcohol was noted in a case of Serejski and Frumkin. One of Wohlfahrt's patients had become subject to violent fits of temper. A patient of Gélineau became more emotional shortly before the appearance of cataplectic seizures six years after the onset of attacks of sleep.

The pugnacity which some narcoleptic patients display on being awakened has been considered. With the exception of two patients whose reactions were psychopathic, none of my patients appeared to be particularly irascible. It seems probable that the change in personality of which irritability is the principal sign rarely reaches a profound degree in narcolepsy.

Although the stubborn nature of the disease frequently causes patients to become discouraged, real attacks of depression are exceptional. A patient who attempted suicide several years before the onset of narcolepsy was subject to attacks of sleep only when depressed. When seen in the clinic he was in a state of euphoria, but such states, which were frequently induced by excitement, ordinarily lasted no longer than a few hours, his usual mood being one of depression. In a recent letter, the patient said that a sense of duty alone kept him from committing suicide. Another patient who had been quite depressed since the onset of her narcolepsy talked of committing suicide. In one of the cases reported through correspondence, the patient had become hopeless and fearful since the onset.

Two other patients had become suspicious as well as irritable; another remarked, in a recent letter, that besides being irritable and depressed, he had become "an awful liar and a coward."

Although mental deterioration seems to be decidedly rare in narcolepsy, an habitually drowsy person is almost certain to be less aware of the events of his environment than one who is wide awake. Löwenfeld's patient said that his memory was poor and that he found concentration difficult; one of Redlich's patients had a poor memory. Morton's patient said that he often felt as though his "mental machinery would not budge." Seven patients who were observed at the clinic complained of impairment of memory, and two others of difficulty in concentrating. A woman described as "flighty" by one of her examining physicians, became much more alert mentally after her symptoms had been relieved by treatment with ephedrine sulphate. The behavior of two other patients was characterized by a certain indifference and lack of interest. The wife of a patient no longer able to support himself wrote that her husband's mental acuity had become reduced and his judgment impaired. Mental and emotional changes observed in post-traumatic cases will be dealt with later.

One of my patients, like Cohen's (39) patient, was inclined to be fanciful. This tendency is probably attributable to the free associations more or less characteristic of the somnolent state.

Morton's patient was a prey to any fantastic ideas during the period of insomnia which preceded the onset of attacks of irresistible sleep. He finally came to believe in the reality of these ideas and to imagine that a great future lay before him. This state of mind did not endure for long, however, and his subsequent behavior seems to have been reasonably normal aside from the tendency to fall asleep on all occasions. His statement that an emotional display would completely unnerve him is somewhat suggestive of cataplexy; since he sternly guarded himself against such displays, however, one may doubt that this patient was actually psychopathic, as Wilson (261) suggested.

Since the general behavior of a patient with narcolepsy is rarely influenced by his fancies or the content of his dreams and hypnagogic hallucinations, the following data are of considerable interest.

A woman, aged thirty-four years, although drowsy, was belligerent during the course of her examination at The Mayo Clinic. According to her husband, a profound change occurred in her personality at the time somnolent and cataplectic tendencies had appeared seven years before. She became suspicious of all those about her and began to neglect her household duties. A year later she became quite like her former self, but five years before admission the irritability and suspiciousness reappeared with the narcoleptic symptoms, and had been gradually becoming more pronounced up to the time of admission.

Fröderberg's patient, an older man, had mild paranoid ideas which seemed to be engendered by nocturnal dreams of an hallucinatory character. Brown's patient, eighteen months after becoming subject to attacks of sleep and cataplexy, as well as to vivid, distressing dreams, began to have hallucinations of sight and hearing; ten months later he became delusional. The mental symptoms later regressed for a brief period, with the narcoleptic attacks.

A girl, aged fourteen years, and of rather inferior intelligence, had shown a tendency to prevaricate a year before examination at the clinic, and two years following the appearance of diurnal somnolence and terrifying nocturnal dreams. Some time later the dreams and hallucinatory experiences began to occur during the day as well as at night, and the patient came to believe in their reality. She insisted on talking about her difficulties, becoming very irritable and impudent when the veracity of her strange tales was doubted. Some of the dreams had recently had a definite sexual coloring. Following the control of diurnal somnolence by the administration of small doses of ephedrine sulphate, the patient seemed to gain considerable insight. This improvement, although much less marked, was still evident when the patient's mother wrote a year later. A few attacks suggestive of cataplexy had occurred in the meantime.

The only diagnosis hazarded in the last case was that of narcolepsy with psychosis. It seemed reasonable, in view of the patient's age and mental development, that her personality was unable to cope with the vivid and terrifying dreams which were the result of a primary disturbance of the function of sleep. The capacity for free association possessed by some narcoleptic patients was believed to have played a part in the elaboration of her weird tales. The possibility of postencephalitic behavior was considered but finally dropped, since the mental symptoms in this case appeared to have been rather closely linked with the patient's hallucinations.

The following extract from Kleitman's review had a direct bearing on the subject under consideration:

"Just as these higher centers are susceptible to the action of poison, they are more susceptible to fatigue. Well-mannered children become cross and unmanageable when they are sleepy. In adults a definite effect of prolonged wakefulness is a poorer performance of these higher centers. The subject is more irritable and unreasonable, less reliable, less honest even, and as the wakefulness continues, he may suffer from hallucinations, or may dream with his eyes open."

In the light of Kleitman's observations, it is astonishing how well most of those suffering from the malady bear up under their load. It would be strange indeed if an occasional narcoleptic patient did not become psychotic. It is possible, of course, that extension of an underlying organic process, as suggested by the progressive course in some cases, may play a part in the genesis of the psychosis.

PROGNOSIS

It has long been known that narcolepsy tends to pursue a chronic course. Redlich concluded that one could "almost speak of an incurable state" and that a certain reduction in the frequency and severity of the attacks was the most to be expected. He doubted that the few who did recover actually had "genuine narcolepsy." Of the twenty-five patients whose cases were studied by Thiele and Bernhardt, only two suffering from genuine narcolepsy and one from posttraumatic narcolepsy improved in the course of years to a degree approaching complete recovery.

Stöcker's patient, a boy aged fifteen years, completely recovered six months after onset of symptoms, and was free of symptoms when Rosenthal (202) examined him fourteen years later. Lhermitte and Nicolas' patient, a girl, aged seventeen years, whose symptoms had existed for about a year, made a satisfactory recovery following spinal puncture; she complained of nothing more than postprandial drowsiness when seen three years later. In the reported cases of narcolepsy without cataplexy, the course seems to have been chronic in most. The sister of one of Parsons' patients, however, ceased to have attacks of sleep following the birth of her first child when she was aged eighteen years, and several members of one of the families investigated by Hoff and Stengel had long remissions from such attacks. In one of Goldflam's cases the attacks ceased fourteen years after onset, to recur in a milder form thirteen years later. Birman's patient had a remission from her sixth to her ninth years. Details will be given later of a few postencephalitic cases in which there was a favorable outcome, as well as of others in which improvement resulted following treatment.

Information obtained, mainly through follow-up letters, relative to the progress of eighty patients with narcolepsy observed at The Mayo Clinic between January 1, 1919, and the middle of September, 1930, has been summarized in table 4. None of these patients had been treated with ephedrine sulphate. A survey of the information obtained in sixty-seven of these cases was published recently by Doyle and Daniels (50). Their classification of the cases according to the degree of improvement shown has been somewhat simplified for this study, and a few of the cases have been placed in different groups.

One of the six patients who died succumbed to postoperative septicemia. A second patient died suddenly at his home a few days after roentgen rays had been applied to the hypothalamic region. The four other patients did not have cataplexy. Two of these complained of obstruction to breathing in the upper part of the respiratory tract.

The Wassermann reaction of the blood of one of the patients was positive. After leaving the clinic, the patient gradually became more sleepy and died six months later, after being confined to bed for only a few days. The evidence in this case seemed insufficient to justify the diagnosis of an intracranial lesion. The other of the two patients just referred to became cyanotic during some of his attacks of sleep. He died of congestive heart failure eighteen months after his dismissal from the clinic. Another of the four patients who did not have cataplexy died suddenly a few hours following tonsillectomy a year after leaving the clinic. The anesthetic agent used was 0.4 per cent cocaine. In the three foregoing cases, attacks of sleep first appeared later in life; all three patients were obese but not excessively so. The last of the four patients who did not have cataplexy died following an abdominal operation performed elsewhere. The brain of any of the six patients was not examined.

TABLE 4

*Course of narcolepsy in eighty cases**

	CASES	AVERAGE DURATION	
		Since onset, years	Since admission, years
Markedly improved	6	8.6	3.3
Somewhat improved (1 without cataplexy)	16	9.2	3.6
Slightly improved (2 without cataplexy)	15	9.6	2.9
Same (6 without cataplexy)	28	11.5	2.7
Worse (2 without cataplexy)	9	10.7	3.4
Died (4 without cataplexy)	6	6.2	1.6

* None of these patients had been treated with ephedrine at the time they were heard from.

Of the seventy-four patients still alive at the time information relative to their progress was obtained, only six (8 per cent) had shown marked improvement; in only two of the six did improvement seem to approach complete recovery. Sixteen (21.6 per cent) were somewhat improved, and fifteen (20 per cent) were slightly improved. Five of the patients listed as slightly improved were better only so far as the cataplectic attacks were concerned. The cataplectic tendency of one of the somewhat improved patients was entirely in abeyance, and improved to a greater degree than the drowsiness in five others of the same group. In one markedly improved patient, on the other hand, the symptoms were confined entirely to an occasional cataplectic seizure. Half of the twenty-eight patients whose condition was unchanged had been suffering from narcolepsy for periods ranging from ten to forty years.

INCIDENCE

Increasing frequency. There has been a rather startling and fairly consistent increase in the number of patients with narcolepsy admitted to The Mayo Clinic in the last ten years. In 1919, one patient was admitted (for the year, 0.0017 per cent of all patients; or 0.05 per cent of neurologic patients); in 1931 there were thirty-three patients (for the year, 0.02 per cent of all patients or 0.4 per cent of neurologic patients). The apparent falling off beginning between 1926 and 1928, as indicated in figure 1 can be accounted for by the average duration of symptoms. Redlich suggested that apparent increased frequency was the result of growing familiarity of members of the profession with the malady. However, important as this factor may be, the experience of such competent observers as Spiller, Bolten, Wilson (261), and

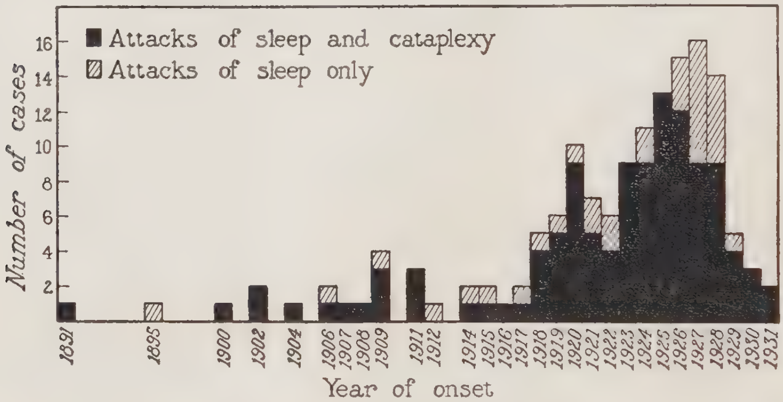

FIG. 1. YEARS OF ONSET OF NARCOLEPSY IN 147 CASES EXAMINED AT THE MAYO CLINIC

In a case listed in the 1928 group as one without cataplexy, it has since been learned that the patient has cataplexy.

Bassoe cannot be ignored. Bolten had noted only two cases of narcolepsy in Holland in the course of thirty years. Wilson, although on the "constant look-out" for twenty years, had not seen a typical example until the few months before he wrote his paper.

Age and sex. The results of my studies of incidence by age are in agreement with the experience of Redlich, Adie, and Thiele and Bernhardt (fig. 2). Of the entire group of 377 patients, 52 per cent had acquired the malady before attaining the age of twenty-one years and 70 per cent before the age of twenty-six years. The only exception to the general rule was found in a group of nineteen men seen at The Mayo Clinic who did not have cataplexy.

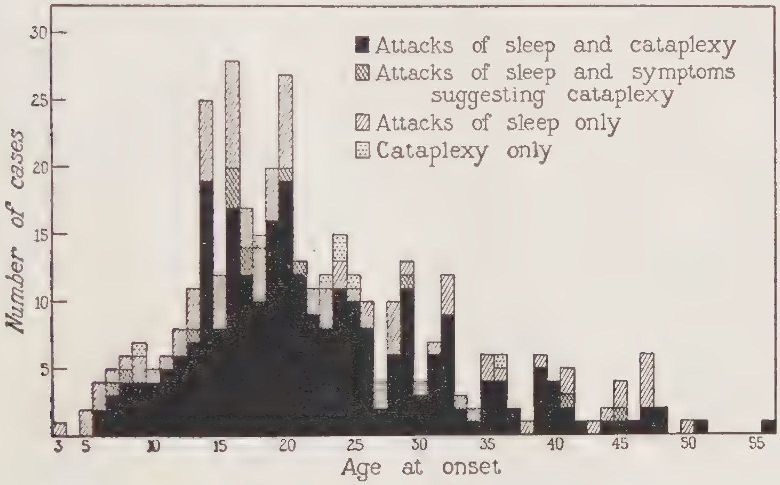

Fig. 2. Age Incidence in 377 Cases of Narcolepsy

It has been learned since this graph was made that the patient whose case was listed in the age eleven group as one with attacks of sleep only, is also subject to cataplexy.

A woman suffering from narcolepsy was formerly looked on as a curiosity, and seventy-nine of the 100 patients in the last series reviewed by Redlich were males. Twenty-three of Adie's fifty patients were women; he expressed the view that the preponderance of males in previous statistics could be accounted for by the number of cases that were observed during the World War. This would hardly apply, however, in the twenty-five observed by Thiele and Bernhardt at the

Charité in Berlin during the last five years. Narcolepsy may possibly place a greater economic handicap on men than it does on women, thus causing a greater proportion of the former to seek treatment. Ninety-seven of the patients seen at The Mayo Clinic were males, and fifty, females.

Geographic situation and race. In the course of a letter prompted by a contemporary reference to African sleeping sickness as a variety of narcolepsy, Gélineau (71) remarked that cases of narcolepsy were observed principally in lands with temperate climates. This statement is yet to be disproved, in spite of the fact that warm weather often seems to aggravate the symptoms of narcolepsy. Holzinger's case of an inhabitant of the tropics is the only one suggestive of narcolepsy that has been reported. No race, however, seems to be immune. Most narcoleptic patients whose cases have been reported in the literature lived in the north temperate zone. Six cases have been reported from Australia (18, 19, 257, 258), one from South Africa (39), and one from Argentina (62). With the exception of a patient suffering from postencephalitic narcolepsy whose home was in Mexico City, a Chinese from Shanghai and a Swede, all the narcoleptic patients seen at The Mayo Clinic acquired the disease while living in various parts of the United States and Canada. Besides Holzinger's case, cases of narcolepsy among negroes have been reported by Thrash and Massee, Wahl, Weech, Hecht, and Freeman, and among Jews by Henneberg and Curschmann and Prange. One of my patients was Jewish. Practically every European race is represented in the reported cases.

ETIOLOGY

Heredity. The evidence in support of the existence of an hereditary tendency to narcolepsy, if due allowance be made for the fortuitous occurrence of narcolepsy in more than one member of the same family, is not very impressive, at least not so far as the number of cases is concerned.

Abnormal drowsiness or attacks of sleep of one of the parents was mentioned in cases of narcolepsy reported by Westphal, Bauer, Maclagan, Jakobsohn, and Hoff and Stengel as well as in two cases observed at the clinic. The father of Bauer's patient became subject to attacks of sleep in youth; the father of Hoff and Stengel's patient, later in life. Parsons reported the onset of attacks of sleep at the time of puberty of two sisters, one of whom recovered a few years later. In one of the families referred to by Hoff and Stengel, four members of one generation and one member of the following generaation were all subject to attacks of sleep during their adolescent years.

In another family investigated by Hoff and Stengel, a grandfather and grandson both had narcolepsy with cataplexy, while in a third family a man subject to cataplexy for many years had two sons, one of whom suffered from attacks of sleep and cataplexy and the other from a neurotic type of sleep disturbance. Of the two brothers whose cases were reported by Rosenthal, only one had cataplexy, but the other had "waking attacks." Narcolepsy with cataplexy of first cousins was noted among the cases at the clinic.

Redlich (198), in reviewing the relatively small number of cases in which a family history of alcoholism, epilepsy, or insanity had been noted, was not impressed with his findings. In my review, I encountered familial psychotic or psychopathic tendencies in twelve cases reported in the literature and in six observed at the clinic; in most instances the psychosis was of the affective type. Members of the patient's immediate family had committed suicide in Curschmann and Prange's case, and in two of the cases studied at the clinic. The incidence of degenerative disease of the nervous system had been high through several generations in Blodgett's case.

Mental make-up. Redlich was of the opinion that evidences of psychopathy were not uncommon among narcoleptic patients. Definite neurotic and psychopathic tendencies were discovered in the histories of only fifteen of the cases reviewed, even if the briefly reported and doubtful cases are included. Ten of the patients at the clinic gave evidence of some abnormal trend, but in several cases these tendencies do not seem to have been especially marked. It must be borne in mind, furthermore, that one inclined to look on narcolepsy as a mark of constitutional inferiority may unconsciously overemphasize the temperamental peculiarities of his patients. Some degree of mental retardation existed in only five of the cases reported and in one case in the group observed at the clinic; it was not at all marked in any. One may reasonably doubt, therefore, that mental and emotional anomalies have any significance so far as the etiology of narcolepsy is concerned, however important they may be in determining the reaction of the individual patient to the disorder. Narcolepsy seems to be rarely associated with any of the usual forms of mental disease; reports of cases in which such an association existed are so brief as to render them of little value for the present study (54, 125, 103). Adie (2), whose patients were not nervous or hysterical in the popular sense concluded that narcoleptic patients do not show hysterical traits more often than other groups in the community. Redlich stated that narcoleptic and hysterical patients seem to have essentially different personalities.

Most of the narcoleptic patients seen at the clinic seem to have been fairly well balanced, as were most of those whose cases have been reported in the literature. Some of my younger patients, like Brock's seemed to possess a considerable degree of self-assurance and were perhaps a trifle superficial. Available evidence is too scanty and altogether too intangible, however, to warrant creation of a narcoleptic personality. Adie's experience would seem to bear out this conclusion, since his patients conformed to no particular mental or physical type.

Physical type. Available information does not appear to warrant establishment of a habitus narcolepticus. Patients at the clinic, like Adie's, did not conform to any particular physical type. Thiele and Bernhardt's patients were predominantly heavy-set, their bodily structure approaching the athletic type in several instances. In Esselevič's four cases, the muscular type was represented three times and the asthenic once. Narcoleptic patients are often overweight, but in the majority of the cases the obese tendency is not evident until the other symptoms of the malady have appeared. Among the 147 narcoleptic patients seen at the clinic, only twenty-three were known to have been obese prior to the onset of narcolepsy; all but four of these were thirty-two years of age or older when they acquired the disease.

I have heard a few physicians comment on the large stature of their narcoleptic patients. In a group of fifty men seen in the clinic who had acquired the malady in the eighteenth year of life or later, the heights ranged from 61 to 74.25 inches (1.55 to 2.08 m.), the average being 68.6 inches (1.74 m.). Eleven were six feet (2.03 m.) or more in height. The heights of twenty-five women who had acquired narcolepsy after full growth had presumably been attained, ranged from 61 to 72 inches (1.55 to 2.03 m.), the average being 64.8 inches (1.65 m.); only one was 6 feet tall.

Epistaxis; headaches. The association of epistaxis with narcolepsy has aroused some interest, although no explanation for the association has been forthcoming. McNamara, in 1862, reported the case of a young girl who ceased to be subject to attacks of sleep following a severe nose bleed. In Mendel's, and Jolly's cases, on the other hand, the patients were subject to attacks of severe epistaxis for a number of years prior to the onset of narcolepsy. The tendency ceased a few years before onset in the latter's case, but in Mendel's case it continued up to the advent of attacks of sleep and cataplexy, as it did in the case of a young man seen by Wilson (262) and Holmes. In one of Wohlfahrt's cases, epistaxis was brought on by severe exertion during adolescence. A history of preceding epistaxis was not obtained in any of the cases of narcolepsy observed at The Mayo Clinic. In ten cases, however, periodic headaches had occurred for many years. In one case, the headaches, which were evidently severe, increased in severity until three years before onset of narcolepsy. In two others the patients began to have headaches a few years before onset.

Preceding infection. Cases in which an acute infectious disease preceded the onset of narcolepsy by an interval of four years or less, have been arranged in tables 5 and 6 according to the time between recovery from the former illness and the first appearance of narcoleptic symptoms. In the clinic cases, preceding infections were assumed to be of some etiologic significance, as a rule, only when the intervals were a year or less. In two of the four exceptions to this rule, a rapid gain in weight occurred shortly after recovery from the acute illness; one patient became subject at the same time to headaches; the preceding illnesses of the other two patients were apparently encephalitic. Of the fifty-five cases listed in table 6, therefore, only forty-three were regarded as instances of post-infectious narcolepsy. In thirty-four of the fifty-six cases listed in table 5, and in eleven of the fifty-five in table 6, the preceding infections were assumed to have represented the acute stage of encephalitis. Although the important question of post-encephalitic narcolepsy will be reserved for later consideration, theses cases have been included in the tables for the purpose of comparison. Illnesses designated in the literature as cases of grippe have been grouped with the influenzas.

TABLE 5

Cases of post-infectious narcolepsy reported in literature

TYPE OF NARCOLEPSY	PRECEDING INFECTION	INTERVAL BETWEEN ACUTE INFECTION AND ONSET OF NARCOLEPSY									
		1 month or less		3 to 6 months		7 months to 1 year		18 months to 2 years		3 to 4 years	
		Cases	Authors	Cases	Authors	Cases	Authors	Cases	Authors	Cases	Authors
Attacks of sleep and cataplexy	Influenza			5	Rosenthal K. Wilson Levin Beyermann (2 cases)	1	Solomon	1	Paskind	2	Spiller Brock
	Encephalitis	9	Stiefler Mankowsky Münzer Strauss Kamman Crouch Tsiminakis Wagner Bolten	5	Wenderowič Stiefler Symonds Kluge Herrmann	3	Adie Strauss D. C. Wilson	3	Maclagan Lhermitte and Roques Bonhoeffer	1	Hoff and Stengel
	Pharyngitis			1	Kahler						
	Febrile jaundice							1	Fulton and Bailey		
	Typhus, typhoid and recurrent fever							1	Serejski and Frumkin		

Doubtful cataplexy	Encephalitis	3	Audo-Gianotti Bolten (2)								
Attacks of sleep only	Influenza	3	Samain Dercum Rosenthal	1	Samain						
	Encephalitis	5	Kraus Perrier (2) Janota	2	Lus Stiefler			1	Lhermitte and Roques	2 1	Lhermitte and Kyriaco Ballet
	Scarlet fever Typhus fever	1	Rybakow								
Cataplexy only	Influenza Encephalitis	1 2	Quenzel Rosenthal Tsiminakis	1	Münzer						

Although influenza has been a more common precursor of narcolepsy than has encephalitis, in the cases observed at the clinic, the reverse holds true for those reviewed from the literature. In three cases in table 6 there were apparently no free intervals between recovery from influenza and the onset of narcolepsy. The infection was complicated by pneumonia in two cases and presumably by typhoid fever in another case. Between 1922 and 1925, inclusive, the proportion of post-influenzal cases admitted to the clinic was high, ranging from 100 per cent in 1923 to 25 per cent in 1925. In spite of the continued increase of narcolepsy, the proportion of post-influenzal cases has been lower since 1925, fluctuating between 3.5 per cent in 1930 and 15 per cent in 1929. Any relation, therefore, if such exists, between the continued increase in the incidence of narcolepsy and the pandemic of influenza, would seem to be indirect. Nevertheless, two of Samain's patients acquired narcolepsy following attacks of influenza during the pandemic of 1892. An attack of scarlet fever preceded the onset of narcolepsy by less than a month in three cases observed at the clinic. In one of the three, in which scarlet fever was complicated by bilateral otitis media, the patient, aged nine years, slept most of the time during

TABLE 6

Cases of post-infectious narcolepsy seen in The Mayo Clinic

TYPE OF NARCOLEPSY	PRECEDING INFECTION	INTERVAL BETWEEN ACUTE INFECTIOUS DISEASE AND ONSET OF NARCOLEPSY					
		1 month or less, cases	1 to 3 months, cases	4 to 6 months, cases	1 year, cases	18 months to 2 years, cases	3 to 4 years, cases
Attacks of sleep and cataplexy	Influenza	4	2	4	4	5	4
	Epidemic encephalitis	6					
	Scarlatina	3			1		1
	Diphtheria	1					
	Measles		1				
	Mumps		1				
	Pharyngitis	2					
	Pneumonia	1				1	1
Attacks of sleep only	Influenza		2	2	2		2
	Epidemic encephalitis	2	1			2	

the year following the scarlet fever and gained 100 pounds. In the course of examination of this patient at the clinic thirty-five years later, she remarked that the attacks of scarlet fever had entirely changed her life, for the narcoleptic symptoms had persisted ever since.

In the absence of any pathologic evidence to the contrary, it seems possible that the acute infection, in the cases under consideration, may have been little more than a precipitant. It must also be emphasized that any actual injury which the brain might have undergone during the course of the infection could have been toxic or degenerative, rather than inflammatory. When the interval between recovery from the acute infectious diseases and the onset of narcolepsy is longer, the former should probably be reported only as a possible contributing facter.

Injuries to the head. The part played by trauma in the etiology of narcolepsy has recently been considered at some length by Rosenthal (204). Since post-traumatic narcolepsy will be considered later, mention will be made here only of those cases in which injuries to the head, if of any significance, appear to have been little more than precipitating or contributing factors in cases in which there was a strong predisposition to the disease. Singer, Goldflam, and Bonhoeffer each reported a single case, and Redlich (198) mentioned two cases in which a more or less trivial injury to the head was sustained shortly before the onset of narcolepsy. Of this group, Goldflam's case and one of Redlich's cases were the only ones in which symptoms directly referable to the trauma were noted. In Fröderberg's case, the time elapsing between the reception of a blow on the head, which occasioned the patient no particular discomfort, and the onset of narcoleptic symptoms was uncertain. Other reported cases in which relatively slight injuries preceded the appearance of symptoms by long intervals will not be considered. Kahler's patient, and one of Bostock's, had sustained fairly severe injuries many years before onset. Gillespie's patient, on the other hand, received a severe injury to his head only twenty-two months in advance of his first cataplectic seizure. Weech's patient sustained a blow on the nose, and Thrash and Massee's a fracture of the nose and superior maxilla, a short time before onset. One of the narcoleptic patients examined in the clinic was rendered unconscious by an injury to his head ten years or more before symptoms appeared.

The interval was much longer in another case, but the patient became subject to frequent headaches about two years after being rendered unconscious for a short time by a fall from a horse. He had also sustained less severe injuries to his head a few years before, and again a few years after the major accident. His headaches gradually became more pronounced, and seven years after their onset, his weight began to increase rapidly. Narcoleptic symptoms developed shortly after an attack of influenza contracted twenty-three years after the major injury. I have heard of a somewhat similar case in which headaches, to which the patient had been subject previously, were aggravated by a blow on the head sustained several years before the onset of narcolepsy. As in the preceding case, the more severe headaches were accompanied by diplopia. In one of my cases, as in one of Ratner's, symptoms appeared to been have aggravated by trauma. In another case at the clinic, the patient was kept in bed by the severity of narcoleptic symptoms which appeared to have been initiated by an injury of moderate severity. Although the patient, then aged seven years, recovered consciousness a few minutes after falling on the ice and was able to get up and walk home unassisted, she remained in bed the following six weeks because her legs, although not paralyzed, gave way whenever she attempted to stand. The patient, who had not been subject previously to narcoleptic attacks, slept most of the time during the six weeks. Other patients whose narcolepsy was not precipitated by trauma, however, experienced a similar acute onset and complained at the time that the legs often gave way.

Endocrine factors. Redlich and Rosenthal, both of whom were impressed by the rapid increase in incidence of narcolepsy at the age of puberty as well as by the relative immaturity of some of their patients suggested the possibility of some constitutional anomaly of the glands of internal secretion being a factor in the pathogenesis. Cases have been reported in which narcoleptic symptoms, attacks of sleep for the most part, were confined more or less to the years of puberty (93, 173, 202). One of two sisters, referred to by Hoff and Stengel, experienced a recurrence of attacks of sleep at the climacteric, the other with each menstrual period. Among the women seen at the clinic, the onset occurred during the first period of menstruation of one and during the menopause of another. The frequency of the attacks increased during menstruation in still another case, as in cases reported by Fischer (60), Ballet, Kahler, and Gruszecka. In Gruszecka's case, the symptoms also became aggravated during pregnancy and lactation. In a case at the clinic, the patient became subject to cataplexy following the birth of her first chlid; the symptoms increased in severity, and attacks of sleep appeared following the birth of a second child. Attacks of sleep were more numerous during pregnancy in another case. Sleeping attacks, furthermore, have occurred only during pregnancy in some cases, and the symptoms of post-encephalitic narcolepsy have apparently been influenced by that state (8, 112, 202).

Wilder reported a case in which attacks of sleep and cataplexy were present for a time following castration. Redlich (198) mentioned one case in which symptoms appeared some time after thyroidectomy had been performed. Esselevič's four patients were said to have presented evidence of hypothyroidism. A low basal metabolic rate and a dry skin were noted in one of Levin's cases, but the patient derived no benefit from medication with thyroid so far as the narcolepsy was concerned.

Over-exertion. Morton's patient and one each of Thiele's, and Curschmann and Prange's, had been subjected to severe physical exertion shortly before the first appearance of symptoms; the period of over-exertion was more prolonged in Kahler's case. Two patients seen at the clinic had, like Jolly's patient, lost much sleep caring for sick relatives during the months immediately preceding onset. Grün's patient, a physician who had been exposed to the rigors of service on the western front for three years, experienced his first symptoms at the age of forty-seven years, during a period of particularly strenuous activity. An older patient seen in the clinic had worked very hard for ten years prior to the onset at the age of forty-one years; one of the patients before mentioned was aged forty-eight years when her symtoms appeared. It is possible that too strenuous activity might precipitate the narcoleptic symptoms of older persons who would otherwise have escaped the malady, although over-exertion, in general, probably only serves to hasten the onset in those strongly predisposed. The debilitating effects of sexual excesses, which were assumed to be the causative factor in Tsitrine's case may have hastened the onset in two of the cases observed at the clinic.

Psychic factors. Some of the patients referred to in the preceding section had no doubt suffered mentally as well as physically. In four of the cases seen at the clinic, as well as in one reported by Gillespie, there was a definite history of mental trauma. In one of the four, the patient was later reported to have recovered following adjustment of certain domestic conflicts, but the motives prompting the report were not entirely disinterested. One of the members of the second family studied by Hoff and Stengel had his first attack of sleep shortly after learning that his betrothed had committed suicide. Brailovsky remarked that many persons in Russia had complained in the last few years of increased diurnal sleepiness, and that some ascribed the symptom to the hardships and uncertainties of the period. Seltzer reported three cases in which narcoleptic attacks were assumed to have been the outcome of marital and other domestic difficulties, but few details relative to these cases are available. Although one of Janzen's patients had similar difficulties, no relation seemed to exist between psychic factors and the severity of symptoms. It seems doubtful that psychic trauma is ever more than a possible contributing factor in cases of narcolepsy.

The assumption of structural alterations within the brain as a result of infection or trauma, although entirely justified in cases in which symptoms appeared following severe injury to the head or a definite attack of encephalitis, is open to considerable doubt in many post-infectious and post-traumatic cases. In the majority of cases there is little if any evidence of a primary endocrine dysfunction; the appearance or aggravation of symptoms at the time of puberty or during menstruation, pregnancy or the menopause is best accounted for by the increased demands placed on the organism at such times. As Rosenthal (204) remarked, it is difficult to conceive of emotional shock or over-exertion precipitating narcoleptic symptoms in one not already predisposed. These reasons and the fact that, in many cases, apparent causes are conspicuous by their absence, compel one to assume the presence of other etiologic factors at present unknown. The onset relatively early in life and the occurrence of disturbances of sleep prior to the appearance of narcoleptic symptoms suggest a constitutional insufficiency of either the sleep-regulating apparatus, or, in Rosenthal's words, the vegetative centers in general. This hypothesis would gain in probability by the reporting of further instances of familial narcolepsy.

SYMPTOMATIC NARCOLEPSY

It was difficult to select cases for study of post-encephalitic narcolepsy, since in many instances the evidence in favor of a preëxisting encephalitic infection was anything but conclusive. I finally selected only those cases in which symptoms suggesting acute encephalitis had been noted during previous febrile illnesses, or in which objective evidence of chronic encephalitis had been demonstrated on general examination. It is probable that I have selected cases that were not encephalitic and rejected others that belong in the group. Somnolence of itself, as Pette remarked, does not permit any definite conclusions to be drawn concerning the nature of the underlying process. Disorders of behavior, in the absence of other evidence of encephalitis, may also lead one astray. Steck found that changes in personality similar to those characteristic of encephalitis, may be observed in other diseases of the brain. It must not be forgotten, furthermore, that drowsiness alone may cause a person to be irritable, that a sleepy child is very likely to be cross and ill-mannered.

Post-encephalitic narcolepsy without cataplexy.—Stern (226) stated that the tendency to excessive sleep and the sense of fatigue, characteristic of the acute stage of the disease may extend into the pseudoneurasthenic or even the amyostatic stages. Diurnal somnolence persisted through the later stages, however, in only 5 per cent of his cases. Stern observed cases in which no objectively demonstrable residue of preceding acute encephalitis remained, but in which the depth and duration of sleep was increased, and the patient was subject to sudden attacks of an irresistible desire to sleep. None of his patients apparently had cataplexy, which be believed was lacking in most cases of this type. He admitted, however, that genuine and symptomatic narcolepsy could be similar. He cited a case in which the somnolent tendency appeared four years after the patient had recovered from the acute stage. Cataplexy was lacking in five of Redlich's seven post-encephalitic cases.

Nine cases of post-encephalitic narcoplesy without cataplexy are listed in table 5. The three cases in which the existence of cataplexy was doubtful have been included in this part of the study as well as five others not included in table 5. In two of the five cases, the interval between recovery from the acute infection and the onset of attacks of sleep was not given; in a third case an interval of six years exceeded the limits of the table. In the two remaining cases there was no history of a preceding infection, although the patients were obviously suffering from chronic encephalitis (126, 257).

Seventeen reported cases of encephalitic narcolepsy without definite cataplexy have been reviewed; eleven of the patients were males and six, females. In thirteen cases in which the age at the time of onset could be determined, narcoleptic symptoms began in the first decade of life in two cases, in the second decade in five, in the third decade in four and in the fourth decade in two. Three of the patients had become obese since onset.

Of five patients with parkinsonism, one also suffered from paralysis of upward gaze and spasmodic laughing (136); another patient presented the residuum of Weber's syndrome (101). In one other case, the signs of chronic encephalitis were not very clear-cut. The duration of the narcolepsy was given in fourteen cases; it was less than a year in two, one year in three, and ranged from two to eight years in seven others. The attacks of sleep ceased entirely two years before in one case and had become less frequent as time went on in three other cases.

The following cases of post-encephalitic narcolepsy without cataplexy were observed at The Mayo Clinic.

A man, aged thirty-four years, was admitted September 19, 1922. He had been rather sleepy ever since he had had typhoid fever twelve years before. Two years before admission he had had another febrile illness, from which he had never completely recovered. During the first two weeks of this illness, he remained in a drowsy or semistuporous state most of the time. Six months before admission, attacks appeared of an irresistible desire to sleep on all occasions. When hitching his team, he would often lean against one of the animals to indulge in a brief nap. He was rather restless at night and had become irritable and depressed. Aside from some irregularity of pupils and considerable hyperactivity of the tendon reflexes, nothing was noted on general or neurologic examination. The patient's spirits improved somewhat after he left the clinic, but in 1928, four and a half years later, he wrote that his condition was about the same. According to a recent letter, this patient died January 20, 1929, following an abdominal operation performed elsewhere.

A man, aged thirty-one years, came to the clinic June 17, 1927, complaining of attacks of sleep which had persisted since an acute illness five years before. Although delirious for five weeks at the time, he could recall having had diplopia over a period of forty-eight hours. He had been compelled to dispose of his business three years prior to admission because he was continually falling asleep when at work. Night sleep was disturbed; he talked in his sleep frequently and had many terrifying dreams. He had gained 41 pounds since onset, and his sexual functions were almost entirely in abeyance. He had become rather irritable. Fixed pupils, a slow gait and a rather expressionless face were noted on neurologic examination. This patient did not reply to a letter of inquiry.

A Mexican, aged twenty-nine years, came to the clinic January 21, 1929, complaining of sleepiness. This symptom had appeared two years before, immediately following an acute illness characterized by insomnia of four days' duration and frequent diplopia. He often fell asleep for a few minutes when reading or in the middle of a conversation, but never when on his feet. Nocturnal sleep was not disturbed and he had lost rather than gained weight. He felt that he was sexually impotent. He had also been aware for some time of gradual slowing in movements and reduction in the speed and acuity of mental processes. Fairly well developed parkinsonism was noted on neurologic examination. In reply to a follow-up letter two years after his admission, he stated that not only was the sleepiness less marked than it had been, but that he had obtained considerable relief from the parkinsonism through the use of scopolamine.

A girl, aged sixteen years, who was admitted June 29, 1929, had been subject to daily attacks of irresistible sleep since an attack of influenza three months before. She slept well at night but had annoying dreams. Although her face was rather expressionless, and movements of the right arm were rather slow, the examining physicians were unwilling to make a definite diagnosis of parkinsonism until they saw the patient a second time, three months later. The parents of this patient have not replied to letters of inquiry.

A woman, aged twenty-five years, who was admitted August 28, 1928, had had influenza three years before admission to the clinic. About a year later she noticed that she could sleep better during the day than at night, but she did not become subject to attacks of irresistible sleep until a year before admission. Her first attack occurred one day when she was teaching school and she had since been having three or four such attacks daily. Improvement in nocturnal sleep coincided with the appearance of attacks of sleep. Memory had become impaired, and the mental processes were slowed during the last year, together with general slowing of movement. On examination, well developed parkinsonism was noted. The basal metabolic rate, as determined on two occasions, was −21 and −16. The patient has not replied to letters of inquiry.

In this group of five cases of post-encephalitic narcolepsy without cataplexy, the sexes were about evenly represented and the patients all fairly young at the time of onset. All but one of the patients presented definite evidence of chronic encephalitis, but only one had gained weight. Unfortunately, only two of this group have been heard from since they left Rochester. One had not improved materially four and a half years after admission and one year prior to his death. The other had improved somewhat two years after admission.

Post-encephalitic narcolepsy with cataplexy. In a group of twenty-four cases of post-encephalitic narcolepsy with cataplexy, reviewed from the literature, seventeen of the patients were males and seven females. The onset of narcoleptic symptoms occurred in the first decade of life in two cases, in the second decade in seven, in the third decade in eight, and in the fourth decade in five. In two cases the patients' ages were not given. In seven cases there seemed to have been no free interval between recovery from encephalitis and the appearance of narcoleptic symptoms; these have been listed in table 5 with two others in which the interval was a month or less. There was some evidence of parkinsonism in twelve cases, but in four the evidence seemed rather slight; in three of the remaining eight cases the parkinsonian symptoms had undergone more or less regression. In three additional cases other residuum of the acute encephalitis was demonstrated. It might be added that some objective evidence of encephalitis was demonstrable in all but one of Thiele and Bernhardt's seven cases of post-encephalitic narcolepsy. Two patients in the group of twenty-four had disturbance of behavior (236, 248). Another patient (Kamman's) was extremely irritable and three others were emotionally unstable. Eight patients had gained weight since the appearance of narcoleptic symptoms. Headache was a rather common feature among the cases reported.

The group of twenty-four cases includes two in which there was perhaps some doubt as to the existence of a preceding attack of encephalitis (137, 259). Two cases, in which somnolence was not evident after the patients had recovered from the acute febrile illness, are listed in table 5 as examples of cataplexy unaccompanied by attacks of sleep. A case of Bolten's and one of Bonhoeffer's are included in the group of twenty-four cases, although neither patient seemed to be subject to the ordinary type of cataplectic seizure. Bolten's patient fell asleep when irritated or amused; Bonhoeffer's patient was subject to the cataplexy of awakening. Gruszecka's case, although included in the group of twenty-four cases, is not in table 5 since the duration of the narcoleptic symptoms could not be determined. Thiele and Bernhardt's seven cases and Redlich's two cases of encephalitic narcolepsy with cataplexy were not reported in sufficient detail to permit their being included in this summary. Lhermitte and Nicolas' case and three classified by Wohlfahrt as encephalitic are likewise not included.

Recovery or considerable improvement is believed to be of more frequent occurrence among patients with post-encephalitic narcolepsy than among those with the essential variety of narcolepsy. In this connection, the five cases in which somnolence ceased with recovery of the patients from the acute stage of encephalitis, or did not persist very long after the appearance of narcoleptic symptoms, deserve special consideration. In Herrmann's case, cataplexy and the attacks of ptosis yielded promptly to administration of strychnine nine months after their first appearance. Rosenthal's patient recovered from the cataplexy three years after onset, and when Mankowsky's patient was last seen two years after onset, the cataplectic attacks had become milder and less frequent. Tsiminakis' patient, however, was still having cataplectic attacks three years after onset, and the duration of the disorder in Wagner's case was too short to permit the drawing of any conclusions. In Symond's case which had some points in common with this group of five cases, the patient was still subject to cataplexy and probably to transitional states when seen six years after onset. Among the remaining eighteen cases of post-encephalitic narcolepsy with cataplexy, the greatest improvement was noted in Stiefler's, and Adie's (1) cases. The attacks in Stiefler's case were greatly ameliorated following two courses of intravenous injections of Pregl's solution of iodine. Adie mentioned two cases of post-encephalitic narcolepsy in which recovery ensued a year after onset. One of the two was probably the case referred to here. Improvement was only temporary in Kluge's case, and that observed in Münzer's case may have been due to the fact that the patient was seen at a time of the year (November) when his symptoms were generally less troublesome. The symptoms had persisted apparently for six years in Strauss' first case; in his second case they had become more marked after an early remission and apparently were not showing further improvement seven years after onset. Wilson's (259) patient showed slight improvement, and Kamman's very little, in the course of eight years. Crouch's patient[3] evidently had made little progress in eleven years. The course of the disease in the last five cases sounds much like that of "essential" narcolepsy.

Conclusive evidence of encephalitis was generally lacking among the 116 cases of narcolepsy with cataplexy observed at the clinic. Five of the cases in which a diagnosis of probable post-encephalitic narcolepsy was made at the time the patients were examined were included by Cave in his post-encephalitic group. Three of the five were post-influenzal cases. The slight notching of the homonymous visual fields in one of these may not have had anything to do with the preceding infection; a slight degree of lateral nystagmus noted in the two other cases was similarly of doubtful significance. A fourth patient had had measles eight years before onset and in addition looked dull and stupid. Only one of the five is included in my post-encephalitic group. In this case the patient had been drowsy during the febrile illness immediately preceding the appearance of narcoleptic symptoms and, in addition, had noticed a tendency to upward deviation of the eyes when she was tired. I do not deny that other post-infectious cases may have been encephalitic. I am not entirely satisfied, on the other hand, that all of the five patients whose histories follow, actually had epidemic encephalitis. As has previously been suggested an acute infection might merely precipitate the narcoleptic symptoms of a patient already doomed to the disease. The transient diplopia mentioned in these cases was similar to that recorded in cases of essential narcolepsy. In one case the severity of onset suggested an acute inflammatory process. Fracassi considered the early hypersomnia, noted in his case, indicative of encephalitis. Similar arguments were advanced in some of the other cases observed at the clinic, the onset in which was acute.

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[3] This case was also included in Partington's and Wagner's reports.

A man, aged twenty-eight years, was admitted to the clinic April 26, 1929; his chief complaint was stomach trouble and sleepiness. In 1918 he had had a rather prolonged illness which was thought to be typhoid fever. Persistent insomnia was replaced two or three weeks after the onset of this illness by a somnolent state that lasted three or four weeks. The patient had been drowsy most of the time for the next eleven years, falling asleep whenever he sat down. His sleep at night was somewhat disturbed, but at the time of his examination a history of cataplectic attacks was not obtained. Nothing of significance was noted on neurologic or general examination. A diagnosis of duodenal ulcer was made on evidence furnished by roentgenologic examination. In a letter dated July 4, 1931, the patient wrote that his condition was about the same, and that any digestive disturbance seemed to increase the sleepiness. His letter also contained the significant information that he was "still bothered with spells of weakness" on laughing. During such spells, which lasted for but an instant, his head jerked and his knees almost gave way.

A man, aged twenty-three years, was admitted to the clinic July 18, 1929. During an attack of influenza in 1918 he seemed to be abnormally drowsy; when convalescing from this illness, he noted transient diplopia on several occasions when he looked up from reading. He had similar attacks later, especially when he resisted the desire to sleep, or looked fixedly at an object. These attacks continued to recur at odd times until he began to wear glasses two and a half years before admission to the clinic. Following recovery from the influenza and its immediate effects, he noticed a tendency to fall asleep whenever he sat down in the evening, particularly when he attempted to read. The tendency gradually became more pronounced until 1926 when he began to have attacks of irresistible drowsiness during the day. Sleep at night became disturbed and he often had terrifying dreams. Fifteen months before admission, an attempt to throw a stick at a dog occasioned his first cataplectic seizure; others followed, but none had been particularly severe. Nothing was noted on neurologic examination except irregularity of outline of the right pupil, which was larger than the left, and moderate horizontal and slight vertical nystagmus. When he returned for treatment seven months later he expressed the opinion that his cataplectic tendency had become less marked, but that otherwise his condition had remained the same. A roentgenogram of the skull revealed a small sella turcica. The patient was partially relieved of his symptoms when taking 50 mgm. of ephedrine sulphate three times daily.

A boy, aged fifteen years, was admitted to the clinic December 29, 1929. A year before admission he had had an illness lasting a week which was characterized by chills, moderately high fever, extreme drowsiness, and numbness of the extremities. He had not felt like doing anything but sleep since recovery from this illness, although he had improved somewhat in this respect during the three months preceding admission. For six months following the illness he had an enormous appetite, and had gained 40 to 50 pounds in the year preceding his admission. He had noticed transient diplopia on a few occasions. The first cataplectic attack occurred a few months after the initial illness. Aside from dryness of the skin, general and neurologic examinations were negative. The basal metabolic rate was −15. February 2, 1931, the patient wrote that although still likely to fall asleep on all occasions he was somewhat better. He has since obtained considerable symptomatic relief from the use of ephedrine sulphate, taken under the direction of his physician at home.

A youth, aged nineteen years, was admitted to the clinic September 21, 1930, sixteen months after an attack of influenza. During this illness, which lasted but a few days, he had a high fever and was very drowsy. He had noticed transient diplopia on one occasion the week following his recovery, and had since been subject to attacks of sleep. When he laughed or became excited he felt "relaxed all over." During the winter following the onset of the narcolepsy he improved, but became worse again the following summer. General and neurologic examinations revealed nothing of significance. The basal metabolic rate was −8. When last heard from, six months subsequent to dismissal from the clinic, the patient said he was much better, but this improvement may have been nothing more than a seasonal variation in the severity of symptoms.

A boy, aged seven years, was brought to the clinic December 7, 1931. He had had bilateral otitis media the preceding April, and pneumonia the following month. About six weeks prior to his admission, he suddenly became ravenously hungry, staggered, complained of headache, and quickly passed into a state of deep sleep from which he could not be roused for four days. Attacks of this nature, always preceded by hunger, headache, partial blindness, and vertigo, had been recurring every two days as a rule. The temperature was said to have varied from 96 to 102°F. When the child was up and around in the intervals between attacks, his mother had seen him fall to the ground on several occasions when he happened to be excited or amused. He had become irritable, rather silly, and difficult to manage. Nothing of note was revealed on neurologic or general examination. When sleepy, the child staggered about, cried, and proved very uncoöperative. Otherwise he displayed a lively interest in his surroundings, played and behaved about as well as the average child of his years. Two attacks of sleep were observed on successive days when the child was in the hospital, his mother's description being confirmed in every detail. The patient had every appearance, as the attack, came on, of a very sleepy child. When in the attack, he could be aroused for the moment only by painful stimuli. As the patient awakened from the first attack in about four and a half hours, the various phenomena noted as the attack came on reappeared. Within ten or fifteen minutes, however, he became bright and active. The second attack was terminated an hour after its inception by an injection of 0.3 cc. of ephinephrine. The patient was then given $\frac{1}{8}$ grain (8 mgm.) of ephedrine sulphate, morning and noon. He had no further attacks during the two days which preceded his departure and the treatment continued to be effective for three months, after which the attacks recurred. The child's temperature remained within normal limits during the four days he was under observation.

In the last year I have observed one case that seemed to meet all the requirements for a diagnosis of chronic encephalitis with narcolepsy. Since the patient was no longer subject to narcoleptic attacks at the time of admission, this case was not included with the others for statistical study.

A man, aged forty-three years, came to the clinic March 2, 1931, complaining of nervousness and weakness. Five years before admission he had had an attack of influenza which confined him to bed for two weeks. Six months later he became subject to attacks of irresistible sleep. As the drowsiness became less marked about eighteen months later, he began to experience some difficulty in bringing his left leg forward. His movements became slower, and about a year later tremor of the right arm appeared. Sialorrhea had been present since the onset of his illness. Although he still felt sleepy at times, definite attacks of irresistible sleep had ceased to occur eight months before admission. Questioning elicited the fact that during the time he was subject to these attacks, embarrassing situations affected him in a peculiar manner. When he came in contact with his employer, for instance, his legs became weak and he would be unable to speak for a few minutes. He was also of the opinion that vision tended to become blurred at such times. Sometimes he would be unable to move for a few minutes just before going to sleep. Although muscular tonus was not greatly increased, the other features of well developed parkinsonism, including facial seborrhea, confirmed the diagnosis of chronic encephalitis.

Encephalitic states bearing some resemblance to narcolepsy. Postencephalitic states, not narcoleptic strictly speaking, but having some features suggestive of that condition, have been reported. Bonhoeffer saw a patient suffering from parkinsonism who had frequent brief attacks in which he could not prevent his eyes from closing. Bonhoeffer believed these attacks represented dissociation of one of the components of normal sleep, an interpretation which seemed to receive some support from the fact that, later in the course of the illness, the attacks tended to come on only when the patient was tired. Although admitting that active contraction of the orbicularis oculi muscles might have played a part, Bonhoeffer was inclined to attribute the attacks mainly to temporary inhibition of the levator palpebrae muscles. In Herrmann's case, attacks of sleep were replaced by attacks of ptosis; Strauss' second patient had similar attacks during which thinking seemed rather difficult. This transient ptosis seems to differ from the usual encephalitic blepharospasm, in that the latter generally recurs more often. Similar inability to open the eyes has been noted during cataplectic seizures and at the onset of attacks of sleep. Bonhoeffer suggested, further, that conjugate upward deviation of the eyes may at times represent dissociation of one of the components of sleep, since the eyes turn up in a somewhat similar fashion during normal sleep. He admitted, however, that in some of the cases of oculogyric spasm, the eyes deviated in other directions. Hall, who shared Bonhoeffer's views, was able, on the other hand, to meet this objection by demonstrating that the eyes do not necessarily deviate upward in states of normal sleep. Stockert found that spasms of convergence and states of unconsciousness and deep sleep could be induced by prolonged ocular fixation.

Comment. Aside from a history of a preceding attack of acute encephalitis and the presence of signs and symptoms of the chronic stage of the disease, post-encephalitic narcolepsy may be similar clinically to the ordinary variety. Münzer considered the tendency to gain weight rather characteristic of the former variety, but this tendency is generally common. Commenting on the relative mildness of the symptoms of encephalitis in many of these cases, Redlich (198) expressed the view that extensive anatomic changes were inconsistent with the production of narcoleptic attacks. Pursuing this thought further, progression of the encephalitic process may have brought about cessation of the narcoleptic attacks in Lhermitte, de Massary and Kyriaco's case, as well as in the case of the man aged forty-three years reported. In the post-traumatic and post-encephalitic cases reported by Papastratigakis, and Willis, respectively, the narcoleptic symptoms became milder with the development of parkinsonism. This suggests that a reduction in the frequency and intensity of the attacks in post-encephalitic narcolepsy may not be an altogether encouraging sign. It is also worthy of comment that with the appearance of narcoleptic symptoms, the reversal of the sleep rhythm characteristic of epidemic encephalitis may disappear.

Since post-encephalitic narcolepsy may be so similar clinically to the ordinary variety, the question arises as to whether the majority of the cases now being seen, actually represent a larval form of epidemic encephalitis. Redlich (198) expressed the view that the incidence of narcolepsy was sufficiently great prior to the pandemic of epidemic encephalitis to render such a position untenable. Two other objections might be advanced: (1) the incidence of narcolepsy seems to be increasing at a time when very few cases of acute epidemic encephalitis are being noted, and (2) in comparison with the more common sequelae of encephalitis, cases of post-encephalitic narcolepsy that can be recognized as such are rare, the subject being dismissed with one sentence in von Economo's monograph. It is possible, of course, that many of the cases of narcolepsy now being observed owe their origin to an attenuated strain of the encephalitic virus which is still propagating itself.

Redlich, in suggesting that other forms of encephalitis might play a part in the etiology of narcolepsy, evidently had in mind disseminated encephalomyelitis. He expressed the view that Lust's case was one of measles encephalitis rather than epidemic encephalitis; further, that Esselevič's case, in which narcoleptic symptoms followed recovery from recurrent fever, belongs to the same group. Redlich had seen a patient who, four months after recovery from the acute stage of encephalomyelitis, became drowsy and subject, apparently, to cataplectic phenomena. This patient had previously had attacks, however, which were similar to Oppenheim's "Lachschlag." It might be added that although cases of encephalomyelitis have become common during the last decade, Redlich's case is the only one of this type on record in which narcoleptic symptoms were associated with objectively demonstrable residue of the acute stage of the disease.

So far as encephalitis is concerned, I would conclude that, on the basis of available evidence, the disease should be regarded only as one of the possible causes of narcolepsy, even though it may possibly have played a major part in the etiology of the more recent cases.

POST-TRAUMATIC NARCOLEPSY

Post-traumatic narcolepsy may properly be considered at this time, not only because it is another variety of symptomatic narcolepsy, but also because signs and symptoms more or less characteristic of the subacute and chronic stages of epidemic encephalitis may appear following injuries to the head (140, 169). I have selected as examples of post-traumatic narcolepsy only cases in which narcoleptic symptoms appeared within a few months following injury sufficiently severe to produce unconsciousness, and other signs and symptoms attributable to a definite injury to the brain. Cases in which trauma appeared to be only a possible contributing or precipitating agent have already been considered. Souques (221), in 1918, reported a case in which attacks of sleep, polyuria, and objective evidence of a lesion in the mesencephalon were associated as sequelae of a severe injury to the head. In a report in 1927 (222) he cited a similar case in which polyuria was lacking, inequality of the pupils being the only demonstrable neurologic sign. Pleocytosis of the cerebrospinal fluid was noted some time after the injury in both instances. Pollock mentioned a case in which the spinal fluid was bloody at the time of an injury; this was followed by attacks of sleep and polyuria. In four cases of post-traumatic narcolepsy without cataplexy reported by Lhermitte, in 1918, two of the patients had been wounded in the head and two others had suffered from "cerebral commotion." Narcoleptic attacks ("type Gélineau") appeared in the case reported by Papastratigakis while the patient was still mentally confused as the result of a recent injury. The parkinsonism and respiratory crises which developed several years later were interpreted as the expression of a progressive lesion initated by the trauma rather than by intercurrent encephalitis. In a case observed at The Mayo Clinic but not included in the 147 cases selected for this study, the patient, a youth, aged nineteen years, had experienced nine attacks of sleep during the fourteen months that had elapsed since he was rendered unconscious for three to four hours by a blow on the head. During these attacks, which were preceded by a sensation of dizziness, the patient could be awakened only with considerable difficulty. The attacks lasted two to three hours as a rule, and were followed by a transient state of mental dullness. Five years after his dismissal from the clinic the patient wrote that he had had no further attacks, and that his general condition was much improved.

Trauma was assumed to be the essential causative factor in three of the twenty-five cases of the complete narcoleptic syndrome studied by Thiele and Bernhardt. One of the three patients concerned evidently showed considerable improvement. In a brief review, Morhardt stated that the course of post-traumatic narcolepsy generally tended toward recovery. Haenel's case seems to be the best example of post-traumatic narcolepsy with cataplexy so far reported in detail. Attacks of sleep and cataplexy were first noted in this case two months after the patient had sustained a severe injury to the head. Changes in personality, circumstantiality, stubbornness, incontinence of affect and increasing irritability distinguished this condition, in Haenel's opinion, from the usual case of narcolepsy. The patient whose history follows was seen in The Mayo Clinic.

A man, aged twenty-two years, came to the clinic November 3, 1931, complaining of drowsiness. When playing football six years before, he had been knocked unconscious momentarily, and although able to continue playing, he remained in a state of more or less complete amnesia for the following week. A certain amount of retrograde amnesia had persisted since the accident, and he had never fully recovered his former mental acuity. When recovering from the immediate effects of the accident, he became subject to attacks of irresistible sleep, which had increased in frequency and intensity during the year preceding his admission. Although laughter had never had any ill effect on him, excitement had caused the strength to leave his arms and legs momentarily. He became irritable and his behavior often showed a general lack of restraint. His mother remarked that, at twenty-two years, his judgment was poorer than it had been during the years of his adolescence. His general health was good. General examination revealed no abnormalities aside from a high degree of nonspecific prostatitis. The cerebrospinal fluid was normal. A roentgenogram revealed no evidence of a previous fracture of the skull, nor could any definite changes be demonstrated by encephalography. Following the latter procedure, however, the patient improved promptly, both in respect to his drowsiness and general behavior.

Comment. Since narcolepsy is a rare sequela of encephalitis or injury of the brain, one is tempted to assume the presence of inherited or acquired predisposition to the disease even in the symptomatic cases. Esselevič expressed the view that symptomatic narcolepsy differed from the idiopathic variety only in that the constitutional factor played a lesser part in the former. Thiele and Bernhardt, impressed by the uniformity of the clinical picture prevailing throughout their post-encephalitic, post-traumatic, and "genuine" groups, concluded that the same pathophysiologic mechanism existed in all cases of narcolepsy. Although the actual mechanism at work is largely a matter of speculation, it seems reasonable to assume that narcolepsy represents a type of reaction capable of being precipitated by a variety of pathologic processes. On the other hand, however unsatisfactory may be such expressions as "idiopathic," "essential," or "disease sui generis," the fact remains that many persons, otherwise in good health, continue to suffer throughout the greater part of their lives, and for no apparent reason, from attacks of sleep and cataplexy.

OTHER CONDITIONS HAVING NARCOLEPTIC SYMPTOMS

Tumors of the brain. As a result of personal experience and an extensive review of the literature, Fulton and Bailey came to the following conclusion: "Tumors involving primarily the hypothalamus may give rise, as initial symptoms, to hypersomnia, diabetes insipidus, adiposity and genital dystrophy." Their second case is of interest to the present study since not only was the period during which the patient had been subject to attacks of sleep (five years) long for a case of tumor, but the patient's account of attacks of weakness in the extremities suggested cataplexy. A typical example of cataplexy, so far as I know, is yet to be described in a proved case of tumor of the brain. According to Lhermitte and Tournay's review, such an association was reported by Alajouanine and Baruk, but Redlich, in reviewing the same report, made a directly opposing statement. The state of "anéantissement" described, in my opinion, is, at the most, slightly suggestive of cataplexy. The sudden attacks described in a case of probable tumor reported by Roussy and Lhermitte were interpreted by Cloake (34) as being cataplectic, but since consciousness was lost during these attacks, this interpretation must be questioned. Hypnagogic hallucinations have been associated with somnolence in cases of tumor and other lesions involving the diencephalon and mid-brain (133). A case reported by Lhermitte and Kyriaco was of interest in that the patient, who was suffering from a tumor of the frontal lobe, was subject to attacks of sleep only during her menstrual periods. Commenting on their second case, Fulton and Bailey remarked that the existence of marked diabetes insipidus, as well as the optic atrophy, would serve to distinguish the case clinically from one of ordinary narcolepsy.

As Weisz has recently shown, tumors in various situations may be productive of somnolence either through direct extension to the region of the third ventricle, or by causing a general increase of intracranial pressure. The symptoms and signs of increased intracranial pressure generally lead to the correct diagnosis in cases of this type.

Syphilis of the central nervous system. Pette, and Redlich (198) had seen cases of syphilitic meningitis, involving the floor of the third ventricle, in which somnolence was a marked feature. In a case of congenital syphilis reported by Ciampi and Foz, somnolence disappeared promptly following institution of treatment for syphilis, and recurred when treatment was interrupted, although tests of the spinal fluid yielded negative results. The following case is of interest in this respect.

A colored boy, aged thirteen years, came to the clinic August 4, 1930, complaining of "sleepy spells." For the last two and a half years he had been falling asleep for short periods five or six times daily; it was often impossible for him to remain awake when eating or shining shoes. The frequency and duration of the attacks seemed to be increasing. The patient was well nourished and well developed, a corneal scar and horizontal nystagmus being the only physical signs of any significance. Wassermann tests (Kolmer modification) of the blood and cerebrospinal fluid were reported as strongly positive. The spinal fluid contained 24 cells for each cubic millimeter. Subsequent efforts to trace this patient have been unsuccessful.

The natural assumption would be that the hypersomnia present in this case was a symptom of syphilis of the central nervous system. That a constitutional factor might have been operative in addition, is suggested by the patient's statement that the grandmother, an uncle, and three cousins, on his mother's side, were subject to attacks of sleep similar to his. Since it was questionable how much reliance could be placed on the story, the case was not included for study with the cases of narcolepsy.

Lhermitte and Kyriaco expressed the opinion that the attacks of sleep complained of by one of their patients were symptomatic of syphilis. Their opinion apparently was supported only by a positive Wassermann reaction of the blood. It was difficult to arrive at a conclusion in regard to one of the cases observed in the clinic. Although the Wassermann reaction of the blood was repeatedly positive and the patient died several months later, the spinal fluid was negative in detail, and aside from irregularity of the pupils, no objective evidence of syphilis of the central nervous system or other organs was discovered. Specific treatment was advised, but it is not known if it was carried out. Drowsiness is marked at times in cases of general paresis. Although the correct diagnosis should be made easily in cases of this type, I have noted one case in which narcolepsy was suggested as a provisional diagnosis. The drowsiness disappeared following treatment with malaria.

Cerebral arteriosclerosis. Adie (2) had noted attacks of sleep in cases of cerebral vascular disease. Although the arteriosclerosis was not severe, the following case was assumed to belong to this category.

A woman, aged sixty-one years, came to the clinic August 10, 1931, complaining of sleepiness of five years' duration, which had become worse two years before. She had never fallen asleep when on her feet, but she had been overcome by drowsiness on numerous occasions when she would have preferred very much to remain awake. Nocturnal sleep was disturbed and she had lost weight. Sclerosis of the peripheral and retinal arteries, as well as hypertension, all slight, were noted on examination.

Aside from cases in which the existence of vascular disease is obvious, the general tendency, in the absence of other apparent causes, is to attribute all cases of hypersomnia which appear later in life, to arteriosclerosis. Against this assumption the same objections might be raised as against the one that almost all of the so-called late forms of epilepsy are vascular.

Diseases of the endocrine glands. Cushing included somnolence among the symptoms of hypopituitarism. In one of his cases, this symptom was comparable to the physiologic state of hibernation, since it tended to recur during the colder months. Narcoleptic patients, on the other hand, are generally worse in warm weather. Of late years the tendency, as expressed by Lhermitte and Tournay, has been to ascribe the hypersomnia observed in cases of pituitary tumor to pressure on the floor of the third ventricle. In the case of Cushing referred to, the tumor lay above the sella turcica. Fulton and Bailey doubted that the hypophysis played an essential part in the production of hypersomnia. Since, as these authors admit, a state of lethargy and lassitude may supervene as a result of lowered metabolism in cases of primary deficiency of the hypophysis, it is possible that the latter may, in some cases, be a contributory factor in the hypersomnia observed in association with tumors of the pituitary gland. Pette expressed a similar view. It might be added that, as a general rule, patients who come to the clinic suffering from tumors of the hypophysis do not constantly fall asleep in the lobbies and examining rooms as do most of those suffering from narcolepsy. In view of Cushing's statement that somnolence is generally a late symptom in cases of acromegaly, the following case is cited.

A man, aged forty-four years, came to the clinic July 14, 1930, complaining of progressive enlargement of various parts of the body. When the change of his features was first noticed eleven years before, he became subject to attacks of sleep which, however, never occurred when he was on his feet. At the same time, he began to have daily headaches which occurred much less commonly three years later. Sexual desire and potentia began to fail eight years before his admission and, for the last three years, had been completely in abeyance. Besides changes characteristic of acromegaly, roentgenograms of the skull revealed that the sella turcica was markedly enlarged. The basal metabolic rate was +16, and there was some evidence of intolerance to heat. A large, lobulated, reddish mass lying anterior to the optic chiasm was found at operation July 23, 1930. The patient passed into coma on the afternoon of the day following operation; he died four hours later. At necropsy a large tumor was found extending from the sella turcica, and displacing upward the floor of the third ventricle. Microscopic study of the growth revealed the presence of many chromaphilic cells.

Patients suffering from myxedema do not seem to be subject, as a rule, to attacks of irresistible sleep. Drowsiness was a prominent symptom in one of the four cases of this disorder that I have observed in the last eighteen months, but it did not appear to have been overpowering at any time.

Pregnancy. Aside from cases previously cited in which pregnancy had some effect on the course of narcolepsy, others have been reported in which attacks of sleep were confined to the gravid state (112). Nevermann reported a case in which the patient became subject to attacks of irresistible sleep in the twenty-fifth week of pregnancy, and recovered completely therefrom following delivery. Kollewijn reported the case of a woman who became subject to similar attacks when pregnant six months. Prompt and complete recovery followed institution of hydrotherapeutic measures. Fraymann mentioned the case of a woman, ten times pregnant, in which somnolence always appeared during the third week of gestation, preceding the first indications of nausea by a week and a half. Adie (2) had seen "sleep attacks in a pregnant women that ceased after delivery."

Diabetes mellitus. In the absence of acidosis, somnolence is a rare symptom of diabetes. Joslin did not mention it in his textbook, and von Noorden and Isaac dismissed the subject with the statement that periodic attacks of somnolence are sometimes observed in cases of diabetes. Naunyn had seen marked somnolence unrelated to diabetic coma in a few cases of both the mild and the severe types. One of Ballet's patients, an obese diabetic patient, improved with treatment, but this case is of questionable significance since it was seen before the modern era of treatment of the disease. In a case reported by

Lhermitte and Kyriaco, however, attacks of sleep, to which the patient had been subject for several years, ceased following treatment with insulin. A case observed at the clinic is of some interest in this respect, although the hypersomnia was a recent symptom.

A man, aged fifty years, came to the clinic December 29, 1931, for treatment of his diabetes. He had never fully recovered normal strength after being ill with scarlet fever five years before. The weakness became more noticeable in the early part of 1931, and in the following summer he became drowsy and began to pass rather large amounts of urine. He continued to work, but had to lie down now and then to take a nap. At times the desire to sleep became practically irresistible. At the time of the patient's admission he was exhausted, drowsy, and mentally depressed. The urine contained a large amount of sugar but no diacetic acid. The blood contained 390 mgm. of sugar in each 100 cc. Five days after treatment with a proper diet and insulin, the urine ceased to contain sugar and two days later the drowsiness disappeared. At the time the patient was dismissed January 5, 1932, he was certain that his drowsiness was a thing of the past.

Redlich (198) expressed the view that both the diabetes and the drowsiness in cases of this type might be the result of a disturbance of the vegetative centers in the tuber cinereum and contiguous parts. This view would hardly apply, however, in cases in which somnolence disappeared following control of the diabetes.

Obesity. Obesity was recognized as a cause of narcolepsy or at least of somnolence by Lhermitte and Tournay, Lhermitte and Kyriaco, Adie (2), and Redlich. The somnolence disappeared entirely following reduction in Sainton's case and became less severe in cases reported by Goldflam and by Lamacq. Münzer's and Günther's patients who were relieved in a similar manner will be mentioned later. Three of Umber's excessively obese patients were so drowsy that they were in danger of falling whenever they ceased moving about. The attacks of sleep occurred less frequently following medication with thyroid in the case of a very obese man treated by Redlich (198). None of the patients referred to here had cataplexy. Among the patients with narcolepsy examined at The Mayo Clinic, prëexisting obesity was more common if they were not subject to cataplexy, so far as the men were concerned. Three women who had cataplexy, however, had been obese before the onset of the narcolepsy. In some cases of the type under consideration, both the narcolepsy and the obesity may be attributable to the same underlying process. In my opinion, somnolence should be ascribed to obesity only when it is relieved by reduction of the patient's weight.

Polycythemia. A few cases of narcolepsy have been cited in which the number of erythrocytes was abnormally high. Lhermitte and Peyre believed that in their case the attacks of sleep and cataplexy were merely symptomatic of the polycythemia but the relations are probably not that simple. Weber, Naegeli, and Brockbank all included drowsiness among the symptoms of polycythemia. Neisser, and Münzer (154) both attributed the somnolence observed in their cases of rather marked polycythemia to deficient oxygenation of the blood. In Neisser's case, attacks of sleep of seven years' standing ceased entirely following venesection. Kraus (114), and Günther each reported a case of polycythemia in which the onset of sleep coincided with the development of an adiposogenital syndrome. A series of cases, reported by Guillain, Lechelle and Garcin, in which relative erythrosis was associated with various lesions involving the hypothysis and tuber cinereum, was believed to furnish evidence in support of the view that erythrosis as well as narcolepsy may be attributable to disturbance in the floor of the third ventricle.

Deficient oxygenation. Münzer expressed the view that the somnolence in his case of polycythemia could be attributed to the presence of a mass of fat in the mediastinum, since the patient was very obese and the cyanosis was confined to the upper parts of his body. The improvement following reduction by means of a proper diet and medication with thyroid seemed to furnish further support for this view. Günther suggested that the polycythemia might be attributable to the same condition although the condition of the blood was not altered materially by reduction of weight in either his case or in Münzer's case. Goldflam ascribed the somnolence observed in one of his cases to marked generalized obesity rather than to a coexisting mediastinal tumor, in spite of the fact that venesection proved beneficial. I have seen one patient with mediastinal tumor, who for the preceding three months, had fallen asleep whenever he sat down. Symptoms referable to the tumor had existed for two years. Physicians at The Mayo

Clinic who have examined many patients with tumor in the mediastinum, however, do not recall one in which the patient was subject to narcoleptic attacks. Lamacq's case was similar in some respects to one of the cases seen at the clinic in which the termination was fatal. Both patients had ankylosis of the jaw, both were obese, and both complained of difficulty in breathing. In Lamacq's case the nasal passages were obstructed by gummas and the tonsils were enlarged. Somnolence is common in cases of Ayerza's disease. Another patient, seen at the clinic, who died of heart failure, had a similar complaint although the only demonstrable causes for his difficulty consisted of a long, soft palate and a short, fat neck. Of the patients subject to attacks of sleep whose cases Rohde reported, one, an obese man, was said to have stenosis of the trachea; another, a boy, recovered from his drowsiness following adenoidectomy.

Umber attributed the somnolence of obese patients he had observed to engorgement of the right side of the heart and the great veins. I had the opportunity recently to examine an extremely obese woman, who, about six weeks prior to her death from congestive heart failure, became subject to vertigo and attacks of an irresistible desire to sleep. Since the patient's face became cyanotic whenever she lay down, compression of the superior vena cava was postulated Although a considerable quantity of edematous fat was found in the mediastinum at necropsy, the brain was not edematous and the pathologists felt that the dilated heart, infiltrated with fat, was the real cause of the patient's difficulties. I do not believe the somnolence in a case referred to in the preceding paragraph could be attributed to the cardiac condition, since when the patient was examined at the clinic three years after the symptoms appeared and eighteen months prior to his death, there was no definite evidence of decompensation. The cyanosis observed during his attacks of sleep was probably attributable to a mechanical factor. It is possible that in certain cases of narcolepsy, metabolic changes may contribute to fatty degeneration of the heart.

Malnutrition. Somnolence may trouble the emaciated as well as the obese patient. In the case of a man, aged thirty-two years, examined in the clinic. the emaciation and lowered basal metabolic rate (−21, −23) were believed to be the result of anorexia nervosa based on erroneous notions about certain foods. Although he often fell asleep when sitting alone, the existence of attacks of irresistible sleep was doubtful. Following institution of a liberal diet and administration of desiccated thyroid gland, the patient improved. A woman, aged thirty-eight years, whom I examined, had become drowsy following complete loss of appetite and the appearance of asthenic symptoms a year preceding her admission to the clinic. Her drowsiness apparently was not irresistible, as it usually is in cases of narcolepsy. She had a relatively low metabolic rate (−15, −18) and was extremely emaciated. Although there was some pigmentation of the skin, a diagnosis of Addison's disease did not seem justified; the final diagnosis was anorexia nervosa. In both of these cases, the possibility of polyglandular deficiency was considered.

Mental deficiency. Berkhan reported the cases of six mentally deficient children who fell asleep frequently during the day. One of these had nocturnal convulsions. Terman found that, contrary to the general rule, the sleep requirements of feeble-minded children did not decrease with age. In some instances, the amount of sleep required increased as the children grew older. A mentally deficient person may sleep because he has no interest in his environment, whereas a narcoleptic patient must often sleep even when he is exceedingly interested in what is going on. One of Levin's patients, a girl whose somnolence had existed since infancy, probably does not belong in this category since her degree of mental retardation was relatively slight.

OTHER CONDITIONS CLINICALLY RELATED TO NARCOLEPSY

Periodic somnolence. Stöcker included in his report the case of a young man, subject to headaches and frequent attacks of epistaxis, who, during the preceding two years, had been sleepy and indifferent for periods of a few days every month or two. The patient fell asleep on all occasions during these short periods which ceased to recur a year later. Kleine had seen five similar cases and was able to review four from the literature. He found that the condition ran a benign course, and that in all cases the onset occurred between the ages of fifteen and twenty years. All patients appeared to be neuropathic or psychopathic, and some of them exhibited behavior disturbances during the periods of drowsiness and apathy.

In one of the cases reported by Levin (119), the patient, a boy, aged sixteen years following an attack of acute tonsillitis became subject to recurrent periods of sleepiness, irritability, restlessness and polyphagia, lasting from one to six weeks. The spinal fluid contained twelve cells. A youth, aged eighteen years, whom I examined, had had four similar attacks of about the same length during the two years preceding his admission to the clinic. The first attack came on shortly after recovery from influenza. The patient was docile otherwise, but was difficult to manage during the attacks and, like Levin's patient, had a ravenous appetite. When seen in the clinic toward the end of a period of somnolence, he appeared dull and apathetic, but was nevertheless restless and would not coöperate very well. According to his mother, he never referred afterward to what had taken place during the attack. Although the patient was evidently of the shut-in type, his behavior was reasonably normal during the free intervals. Treatment with ephedrine sulphate by his physician at home, had had no observable effect on the somnolence. General physical and laboratory examinations revealed nothing of any consequence. Spinal puncture was not made. In Campbell's case, and in one of those reported by Tsiminakis, the periodic states of somnolence following acute infections, epidemic encephalitis in the former and dengue fever in the latter, had certain features suggestive of catatonia.

Somnolence in neuropathic or psychopathic patients. Bratz and Rohde both included in their contributions cases in which rather prolonged sleep had followed states of emotional tension induced by unpleasant experiences. Some of Brailovsky's cases and one case of Singer's were evidently of this type. In Singer's case, certain features of the patient's attacks, suggested an hysterical twilight state; this would have been a better term perhaps for states referred to by Parmentier as the narcoleptic form of hysterical sleep. There seems to be little doubt, on the other hand, that certain persons of a neurotic or psychopathic make-up pass into a state of actual sleep under circumstances involving considerable emotional tension. Laudenheimer reported a series of cases in which states of prolonged sleep served as a means of escape from unpleasant situations. In the usual case of narcolepsy, sleep is generally not so prolonged, and when it is actually induced by affect, which is not very often the case, the emotion responsible usually is pleasant. Gélineau's first patient furnished a striking example of this.

Conditions related to cataplexy. The attacks designated as "Lachschlag" by Oppenheim were perhaps more closely related to epilepsy than to narcolepsy, since the patients were apparently unconscious during the attack. In the latter paper Oppenheim (168) said that he was not familiar with Gélineau's work at the time he published the former report. Although he felt that his Lachschlag was related to narcolepsy, he did not believe that the two conditions were identical. Rothfeld reported a case in which attacks of a cataplectic nature were precipitated both by laughing and orgasm. Since the patient was said to lose consciousness in some of the attacks, the case may have been similar to those reported by Oppenheim. Rothfeld used the terms "geloplegia" and "orgasmoplegia." Levin (121) used the term "aphonogelia" in reference to the case of a young woman who never had been able to laugh audibly.

The appearance of attacks of powerlessness on awakening, apart from other symptoms of narcolepsy, is interesting because of the obvious relation of the phenomenon to cataplexy. Pfister stated that he had seen twenty examples of disturbance at awakening which he termed "delayed psychomotor awakening." He had noted it only among epileptic persons or persons with some neuropathic or psychopathic taint. He expressed the view that in sleep the normal connections between the psychic processes and motility were loosened, and that persistence of this state, after the patient had awakened, was responsible for delayed psychomotor awakening. Rosenthal (202) mentioned a patient who was subject to jacksonian seizures in addition to waking attacks. According to Wilson, the type of attack under consideration was one of the varieties of nocturnal paresis or paralysis studied by Mitchell. Wilson had seen several examples of the condition which he did not regard as "anything else than a transient physiological disorder." Lhermitte and Tournay expressed the opinion that it bordered on the physiologic state. Two patients complaining of cataplexy of awakening, whose cases were reported by Lhermitte and Dupont, were subject to anxiety states. A woman of neurotic make-up whom I examined had experienced the phenomena on several occasions.

States of morbid dissociation. Rosenthal included attacks of sleep and cataplexy in a larger group of states of morbid dissociation. The other two conditions included in the group, somnambulism and waking attacks, were regarded by Rosenthal as corresponding opposites. In the former, the motor and static functions awakened while the psychic remained asleep, the reverse being true of the waking attacks. As stated, Rosenthal contrasted the attacks of sleep, in which the patient continued to walk, and cataplexy, in a similar manner. If the group under consideration was extended to comprise states of intrapsychic dissociation, it would include hypnogogic hallucinations, which occur apart from narcolepsy, usually among nervous or psychopathic persons (133, 146), and the "Schlaf trunkenheit" of the Germans. The narcoleptic patient seems peculiarly susceptible to all manner of disturbances of sleep.

Relation of narcolepsy to epilepsy. As narcolepsy is a paroxysmal disorder, its possible relation to epilepsy has always aroused interest. So far as the general run of cases is concerned, the attacks of sleep, from which the patient can be aroused as from normal sleep, and the cataplectic seizures, in which consciousness is not lost, are both so different from any of the usual manifestations of epilepsy that mistakes in diagnosis are easily avoided. The patient's description of intermediate or trance-like states may suggest petit mal, since consciousness is not always totally abolished in the latter, but the association with attacks of sleep and cataplectic seizures should remove all doubt as to the true nature of the disorder. The rule that narcoleptic attacks are generally less abrupt and of longer duration than petit mal, however, has exceptions.

Those who are opposed to the view that narcolepsy is something distinct and apart from epilepsy find some support for their position in the occasional occurrence of narcoleptic attacks of patients subject to epileptic seizures. Attacks of sleep, for instance, occurred in addition to convulsive seizures in three cases reported by Fere and in one each of Rousseau's and Gowers' cases. André-Thomas' case, one of Jacoby and one of Worster-Drought probably belong in the same group. Goldflam and Redlich (198) each reported a case in which for a number of years prior to the appearance of convulsions, the patient was subject to frequent attacks of sleep. The order was reversed in another of

Redlich's cases, and in Heuyer's case sudden attacks of unconsciousness ceased to recur three years prior to the onset of sleeping attacks. In Mendel's case, the patient was subject for a number of years to daily seizures of epistaxis, not to epileptic attacks as Serejski and Frumkin asserted. Attacks more or less suggestive of cataplexy have been noted prior to the onset of convulsive seizures and after the subsidence or control of such seizures (38, 79). Edel and Wilson (260, 262) both cited several instances of the association of cataplectic and epileptic phenomena. A few, at least, of the cases just referred to might serve to refute Redlich's statement that a typical cataplectic seizure had never been known to occur in a case of epilepsy. Redlich mentioned cases reported in the literature, as well as one of his own, in which epileptic attacks were precipitated by emotion. Zádor's case probably belongs to the same group.

Wilson did not approve of the tendency of most modern writers to regard epilepsy and narcolepsy as separate and distinct entities. In discussing the subject before the Royal Society of Medicine, he said: "I think, moreover, that the resemblances are greater than the differences, and that we have here a pair of corresponding opposites in a Jacksonian sense." In his paper on "The narcolepsies" he wrote: "So far as the preceding considerations take us, they make out a *prima facie* case for the possibility of some of the narcolepsies and the epilepsies having a common origin." Cave shared Wilson's belief that in the present state of knowledge, final judgment should be suspended. By 1927 Redlich was ready to admit that narcolepsy and epilepsy, though different clinically, might possibly be related from the standpoint of pathogenesis. In his last contribution he considered at some length the possibility of irradiation of the process in the one condition causing symptoms of the other to appear. He made an assertion, however, that was essentially the same as the following one made by Adie (2): "No patient with these symptoms (attacks of sleep and cataplexy) has ever become epileptic in the ordinary sense although some of them have been narcoleptic for twenty, thirty, in one case forty, years." It might be added that an exceedingly small number of persons subject to attacks of sleep alone has ever become epileptic. Basing their opinion on the character of the dreams, the patient's behavior on awakening, the changes in character, the lowered tolerance to alcohol as well as the history of somnambulism in childhood, Serejski and Frumkin regarded their cases as instances of epilepsy expressing itself in the narcoleptic syndrome. In Redlich's opinion their argument was not very convincing.

The failure of narcoleptic attacks to respond favorably to treatment with bromides and other sedatives was used by Gélineau and more recent authors as an argument against the epileptic nature of narcolepsy. In recalling that in Gowers' case, the convulsive seizures but not the attacks of sleep yielded to administration of bromides, Wilson questioned the validity of deductions based on the results of treatment. Janota suggested that the unfavorable effects of phenobarbital in cases of narcolepsy might serve as a means of distinguishing that condition from epilepsy, particularly from petit mal. Regardless of the fallacies inherent in conclusions based on the effects of treatment, two facts are worthy of consideration; first, the two major symptoms of narcolepsy, in many cases at least, are controlled by a powerful stimulant, ephedrine, and second, the most serious manifestation of epilepsy, grand mal, is best treated with sedatives.

Since certain diseases of the brain capable of inducing epileptiform seizures are apparently associated in some cases with narcoleptic phenomena, it is not surprising that symptoms suggestive of both narcolepsy and epilepsy may be encountered now and then. Although epilepsy is more commonly associated with tumor of the brain and post-traumatic states, narcolepsy is more commonly associated with epidemic encephalitis than is epilepsy. Lesions confined to the region of the hypothalamus, furthermore, are probably more likely to produce narcoleptic rather than epileptic symptoms. In the general run of cases of narcolepsy, on the other hand, the possible etiologic factors, when such can be found, are of doubtful significance, this being true of many cases of epilepsy. It is often impossible to distinguish clinically the so-called idiopathic forms of the two syndromes from the symptomatic forms.

In view of its distinctive clinical manifestations and its apparently less serious nature, however, it would seem advisable to regard narcolepsy, for the time being at least, as something distinct from epilepsy. That certain states which seem to represent a transition between the two conditions are occasionally encountered no one can deny, but, as

Redlich remarked, many diseases have maintained their independence in spite of such transitions. So long as the essential nature of the two conditions remains in the realm of hypothesis, it is better to rely on the results of clinical observation. For some time to come, I believe, most students of narcolepsy will agree with Adie (2) that "epilepsy is hardly the best label for patients with sleep attacks and cataplexy only."

DIAGNOSIS

Given a knowledge of the characteristic symptoms, the diagnosis of narcolepsy should present no difficulties. If the cataplectic tendency is not particularly marked, some difficulty may be encountered in getting the patient to describe an attack. Leading questions should be avoided, since a description in the patient's own words is much more convincing than a story giving evidence of suggestion on the part of the physician. In the absence of cataplexy, it is necessary for the examiner to convince himself that the desire to sleep is actually irresistible on some occasions at least. This may not be an easy matter since some narcoleptic patients are inclined to minimize their difficulties. In some instances, information obtained from friends and relatives of the patient is of considerable value. Although the likelihood of serious underlying disease, even in the absence of cataplexy, is not great, a complete physical and neurologic examination, including ophthalmoscopy, is always indicated. In the differential diagnosis, tumor, syphilis and other diseases involving the brain and surrounding structures must be considered in addition to epilepsy, endocrinopathy, psychoneurosis and polycythemia.

In the absence of cataplexy, I prefer the diagnosis of "narcolepsy without cataplexy" to "morbid somnolence." If signs and symptoms of chronic encephalitis are present, the diagnosis, "chronic encephalitis with narcolepsy," is better, in my opinion, than merely "chronic encephalitis." If no residue of an alleged acute attack of encephalitis can be demonstrated, the diagnosis should be "post-encephalitic narcolepsy"; if there is some doubt as to the nature of the preceding infection, "narcolepsy, probably post-encephalitic."

TREATMENT

Complete disappearance or marked amelioration of narcoleptic symptoms has followed, in a few instances, both the intrathecal injection of air and the mere withdrawal of cerebrospinal fluid. In Lhermitte and Nicholas' case, the latter procedure was effective, whereas in a case observed in the clinic the patient's headaches and his attacks of sleep were completely relieved by withdrawal of spinal fluid although the cataplectic tendency persisted. The attacks disappeared following the same procedure in a case reported by Lhermitte and Roques, but recurred in a milder form eight days later. Since the fluid was under increased pressure and since spinal puncture occasionally had been effective in diabetes insipidus and diabetes mellitus, Lhermitte and Roques advanced the hypothesis that withdrawal of fluid might possibly have "modified the functional or dynamic state of the vegetative centers," in the floor of the third ventricle. Of the many narcoleptic patients who have submitted to lumbar puncture, however, few, as Redlich (198) remarked, have benefited therefrom. Benedek and Thurzo saw marked improvement follow repeated injections of small amounts of air into the cisterna magna, but the period of observation was too short to warrant drawing any definite conclusions. In my case of post-traumatic narcolepsy the symptoms were almost completely relieved following encephalography, although treatment with ephedrine sulphate had been of little benefit previously. When last heard from nine months after his dismissal, the patient was still free of his abnormal drowsiness. In another case the partial relief which followed encephalography persisted for only a few weeks. The procedure does not seem to have been of any therapeutic advantage in one of Wagner's and seven of Thiele and Bernhardt's cases. Thiele and Bernhardt stated in their complete report that in their post-traumatic cases, one patient was completely relieved following encephalography. In the remaining cases, one patient was partially relieved, and two others were temporarily relieved by the procedure.

Of nine patients treated in the clinic by irradiation of the hypothalamic region, two were somewhat improved when last heard from. One died a few days after receiving the treatment. Doyle and Daniels (50) made the following statement in regard to the third patient; "He had received treatment by roentgen rays over three fields, a frontal, a right temporal and a left temporal. The dose used was about three-fourths of an erythema dose on the surface, and by cross-fire should have produced approximately 60 per cent of the surface dose in the region of the floor of the third ventricle. Although it is well known that the hypothalamic region is very resistant to irradiation, it is conceivable that some latent process may have been activated to the point at which it could have produced death. Such an assumption, however, would be purely speculative." Commenting on the same case, Cave wrote: "The supposition of a hemorrhage into a rapidly growing neoplasm softened by the x-ray therapy is not an impossibility." The long duration of symptoms, however, was hardly consistent with the presence of a rapidly growing tumor. Six other patients were evidently not helped by irradiation. Redlich, who was apparently the first to recommend the procedure in cases of narcolepsy, had never seen any particular benefit result from its use.

From the reviews of Doyle and Daniels (49), and Mouzon, it is apparent that prior to the introduction by Janota of ephedrine sulphate as a means of symptomatic relief, the results of the symptomatic treatment of narcolepsy were anything but consistent. Treatment with sedatives generally has not been efficacious; the attempt to increase the amount of nocturnal sleep with the use of barbital has in some instances increased the diurnal drowsiness. Four patients examined in the clinic obtained some benefit from the use of caffeine, but six others did not. Benefit has occasionally been obtained from the combined use of stimulants and sedatives (18, 19, 92). The oral administration of preparations of thyroid gland, although the most successful of the various measures employed by Redlich (198), afforded some relief to only seven of twenty patients so treated at the clinic. Treatment with preparations of pituitary gland, effective in four of Beyermann's and one of Ratner's cases, was without value in five cases in which treatment was given at the clinic. A ketogenic diet afforded marked relief in one of Solomon's cases.

There is no question but that some patients with narcolepsy, with proper encouragement, may learn not only to resist the somnolent and cataplectic tendencies to better advantage, but to modify their habits in accordance with the demands of their infirmity. Even then, however, they are never free from the constant threat of being overcome. After exhausting his therapeutic armamentarium, Gélineau concluded that the bolstering of the patient's morale with psychotherapeutic measures offered better prospects than any other form of treatment. Loewenfeld and Weisenburg both had similar experiences. Villaverde found mental suggestion of benefit in the case of a boy, aged ten years, subject to cataplexy but not to attacks of sleep. Psychotherapy had never been beneficial in Redlich's experience. Missriegler claimed to have effected a cure by psychoanalysis, but his description of the symptoms leaves the reader in doubt as to whether the patient concerned had narcolepsy.

TABLE 7

Results of treatment with ephedrine sulphate in seventy-six cases

TYPE OF NARCOLEPSY	RESULTS	DURATION OF TREATMENT						
		Few days	1 to 2 months	3 to 4 months	5 to 6 months	7 to 8 months	12 to 14 months	Total
Attacks of sleep and cataplexy	Satisfactory	8	5	10	2	5	6	36
	Satisfactory but treatment not regular				1		3	4
	Fairly satisfactory	1	2	2	1	2	3	11
	Benefit limited largely to cataplexy				1		2	3
	Not lasting			2	3		2	7
	Poor	2	2	2				6
Attacks of sleep only	Satisfactory	4			2	1		7
	Poor		1	1				2
	Total	15	10	17	10	8	16	76

According to Janota, who demonstrated the value of treatment with ephedrine sulphate in two cases, Skala used the drug in five other cases with consistently good results. Grün, on the other hand, found the treatment of no value in one case. In table 7 are summarized the results obtained in a group of seventy-six patients who were treated with ephedrine sulphate prior to January 1, 1932. The results of treatment in forty-six of these cases up to August 1, 1931 was reported by Doyle and Daniels in 1932. The plan of treatment generally followed in the entire group of seventy-six cases has been to begin with the oral administration of a capsule containing 25 mgm. of ephedrine sulphate before breakfast, before lunch and at 4:30 p.m. If the patient is not entirely relieved, the morning and noon doses are increased to 50 mgm., but the late afternoon dose is kept as low as possible, and in some instances omitted, in order to avoid disturbing nocturnal sleep. A few patients have taken as much as 75 mgm., whereas others have obtained relief from 16 mgm. A recent patient, not included in the seventy-six, who was greatly upset by ordinary doses, was completely relieved of her symptoms when taking 8 mgm. Few patients have complained of any unpleasant effects aside from transient palpitation or tingling in the extremities.

In forty-seven of the cases included in table 7, treatment had been instituted at The Mayo Clinic; twenty-two of the twenty-nine patients treated elsewhere had been examined in the clinic, the remaining seven cases were made known to me through the courtesy of the attending physicians. I have used the expression "satisfactory," in classifying the results of treatment, instead of "complete relief" because it is difficult to establish the criteria of complete relief. A certain amount of diurnal drowsiness is not abnormal, and some patients when taking ephedrine sulphate will be overcome with sleep only when they are fatigued. The patient who never neglects to take his prescribed dose of ephedrine sulphate is exceptional; some, wisely perhaps, prefer a daily nap to an extra dose of ephedrine. "Fairly satisfactory" as used in the table implies a substantial reduction in the frequency and depth of the attacks. The results are classified as not lasting in cases in which the treatment ceased, after a time, to afford much relief, and as poor in cases in which little if any benefit was obtained.

Although little more than symptomatic relief was ever expected, the treatment appears to have been productive of more lasting results in two of the cases included in table 7. One patient found the drug no longer necessary six months after she had begun to take it. In the following six months she had noted no recurrence of symptoms aside from moderate postprandial drowsiness. The other patient, after being completely relieved for a few weeks by small doses of ephedrine sulphate, submitted to tonsillectomy. As the symptoms failed to return following the operation, the patient did not resume medication; there had been no recurrence when the patient was last heard from two months later, although she had been drowsy most of the time for ten years prior to the beginning of the treatment.

In general, ephedrine sulphate suppressed the cataplectic tendency more effectively than the drowsiness. In three cases the benefit was limited largely to the cataplexy, although all three patients were more wide awake in the intervals between their attacks of sleep and felt better generally. One of the three patients slept better at night as a result of treatment, but another reported that his nocturnal sleep continued to be disturbed.

In the cases observed in the clinic, and those that have been reported in the literature, eighty-four narcoleptic patients are known to have been treated with ephedrine sulphate prior to January 1, 1932. If only the cases in which the results were poor or not lasting are excluded, treatment was distinctly beneficial to sixty-eight of the eighty-four patients. If more patients had been traced for longer intervals, the results in all probability would not have been quite so good. Although some patients doubtless acquire increased tolerance to the drug, progression of the underlying process itself may also be a factor in those cases in which treatment with ephedrine sulphate ceases after a time to be effective. Most narcoleptic patients are delighted with the benefits they obtain from the use of ephedrine, which is generally effective from the first dose. This treatment has, of course, the weakness inherent in all forms of symptomatic treatment, but most patients continue to take it, if only when their infirmity is likely to occasion them considerable difficulty. More patients would take the drug regularly if it were not so expensive. Early in my experience with ephedrine, the thought that the somnolence might represent a response to some real need of the patient's organism occasioned me some anxiety, since if that were true, treatment with ephedrine might prove harmful in the end. At present, these fears do not seem to have been well founded, although a few of the patients reported that their symptoms were more pronounced than formerly if they neglected to take the drug. The faint hope that prolonged treatment with ephedrine might effect a rebalancing of the disordered sleep-regulating mechanism, on the other hand, has received some confirmation.

In considering the possible mode of action of the drug, Janota (101) referred to the work of Morita who found that although ephedrine counteracted the effect of chloral hydrate in decerebrate rabbits, caffeine failed to exert an antihypnotic effect under similar conditions. Janota concluded, however, that in addition to stimulation of the subcortical sleep regulating centers, the beneficial effect of ephedrine might also be attributable to direct action on the cortical mechanisms involved in the production of sleep, as well as to an indirect effect through stimulation of the vegetative nervous system. Vondracek suggested that since parasympathicotonia predominated during sleep, ephedrine might exert its sleep inhibiting powers through its known action on the sympathetic system.

A CONSIDERATION OF THE NATURE OF NARCOLEPSY

Although several of the conditions, with which somnolence may be associated as a symptom, are more or less serious, there appears to be no evidence that the symptom in itself is injurious to the organism. Narcolepsy, furthermore, does not appear to be inconsistent with a long and healthy life. Since sleep is primarily a restorative function, it is not unlikely that the excellent general health of so many narcoleptic persons may be referred, in part at least, to their frequent naps. Aside from the danger of accidents, the obese tendency, which is often not particularly pronounced, appears to be the only real threat to the patient's health so far as the narcolepsy itself is concerned. The restorative functions of rest and nutrition are probably interconnected in various ways. Rosenthal, for instance, was impressed by the points of similarity existing between the cycles of sleep-waking and of hunger-repletion. Aside from the favoring influence of frequent naps on the general state of nutrition, the increased appetite no doubt contributes in some cases to the increase in weight. Considerations of this sort call to mind the biologic theory of sleep propounded by Claparède and introduced by Camp into a review of the nature of narcolepsy.

Claparède regarded sleep as an instinctive reaction, since to him it represented a means of defense against intoxication from the products of fatigue. "Psychologically," he wrote, "sleep consists in a lack of interest in the existing exterior situation, and this lack of interest corresponds physiologically to a reflex of inhibition. So far as the restorative function of sleep is concerned, it would seem to be due to an augmentation of 'vegetative tension' rendered possible by a liberation of 'mental tension'."

Camp agreed with Rybakoff that narcolepsy is symptomatic of neuropsychic degeneration. The desire for sleep, he reasoned, could, like the desire for food, become perverted, since both types of desire, although usually indicative of an organic need, are not necessarily indicative. Camp concluded that narcolepsy represents an impulsion to sleep just as bulimia might represent an impulsion to eat. Missriegler expressed the opinion that in his case the narcoleptic attacks permitted the patient to reëxperience previous sexual trauma as well as certain criminal phantasies. The attacks further served, in his opinion, to prevent the translation of the phantasies into reality. Although Missriegler did not deny that the symptoms might have owed their origin to an attack of encephalitis from which the patient recovered shortly before they appeared, he argued that symptoms of an organic disease could be drawn into a parapathic (neurotic) system. The fact that the libido may find expression in the dreams of some narcoleptic patients would not, however, warrant any general conclusions relative to the etiology of the syndrome. Few modern writers have looked on narcolepsy as a form of neurosis. Gélineau probably did not use the term in its present restricted sense, since in his day any neurologic condition without known anatomic basis, such as idiopathic epilepsy, migraine or chorea, was often referred to as neurosis. Redlich, in classifying narcolepsy as "neuropathy with organic characteristics," probably had a similar group in mind.

Although the intensity of the narcoleptic patient's desire to sleep cannot be questioned, it is generally recognized that primitive desires have a physicochemical basis. Discussions as to whether narcolepsy is an organic or functional disease, however, are more or less fruitless. As Cobb has recently remarked, the term "organic," as generally employed, merely implies the presence of abnormalities that are discernible by methods of anatomic study available to us at present.

Although anatomic proof is lacking, experience with tumors and other lesions involving the hypothalamus has permitted the drawing of tentative conclusions relative to the region of the brain involved in cases of narcolepsy. Adie and Rosenthal, in agreement with Redlich, expressed the belief that the syndrome was to be attributed to a disturbance of the pituitary-diencephalic system. Von Economo (51) thought it probable, although not proved, that the malady had its primary cause in a yet unknown disease involving the sleep-regulating center, which he believed was situated in the anterior region of the aqueduct and the posterior wall of the third ventricle. In the light of recent experience, it seems possible that the hypothetical lesion could be the result of trauma and various infections as well as defective development. Cloake, in his review of the influence of the diencephalic centers on metabolism, expressed some doubt as to the diencephalic origin of narcolepsy. His statement that metabolic disorders such as occur in diseases of the diencephalon are not seen in idiopathic narcolepsy must be questioned, even though polyuria is rare, sexual disturbances relatively uncommon and the tendency to gain weight by no means constant. The fact that metabolic disturbances are generally less pronounced in narcolepsy than in known organic diseases involving the centers in question, would indicate that the hypothetical lesion responsible for the former condition was relatively mild.

Trömner expressed the opinion that narcolepsy furnished one of the best supports for his belief that regulation of sleep is a function of the thalamus. He assumed that the inhibitory process always originates in that center, passing to the cortex in the attacks of sleep, and to the striopallidal system in the cataplectic seizures. Rosenthal (202) had previously suggested that the discharge originating in the vegetative centers in the floor of the third ventricle passes to the thalamus and there prevents afferent impulses from reaching the cortex, the result being an attack of sleep. In order to account for the cataplectic seizure, Rosenthal postulated a functional weakness of the thalamus which renders it unable properly to direct the flood of impulses under conditions of emotional stress. He saw some support for his hypothesis in the paresthesia that accompanies some of the attacks. The influence of affect on muscle tonus also caused Redlich and Adie to include the thalamus and other subcortical centers in their theoretic considerations.

Mankowsky offered two hypotheses, suggesting first the possibility of sudden exhaustion under emotional stress of the tonus-regulating mechanisms of the cerebellum and midbrain, second, disturbance of the vegetative centers in the floor of the third ventricle which was assumed to upset the ionic equilibrium or to interfere with muscular function in other ways. The points of similarity between periodic familial paralysis and the cataplectic state, in his opinion, furnished some support for this second view. Doyle suggested, during the course of our work with ephedrine, that the favorable action of the drug, in cases of narcolepsy, might be exerted peripherally. According to an hypothesis of sleep proposed by Kleitman, fatigue leads to muscular relaxation, which in turn, as the result of diminution in the number of proprioceptive impulses flowing toward the brain, promotes sleep. In this way Mankowsky's hypothesis, which was intended to apply only to the cataplectic seizures, might be extended to account for the attacks of sleep.

The general opinion, as expressed by Rosenthal, is that either the primary disturbance in the vegetative centers may exert some nervous influence on the hypophysis, or that, conversely, the latter structure may exert some hormonal influence on the diencephalic centers. Beyermann expressed the opinion that, in some cases, narcoleptic symptoms might be attributable largely to hypopituitarism. This opinion was based on the hypersomnia observed in some cases of pituitary tumor and the anomalies of the sella turcica encountered in cases of narcolepsy, as well as on the beneficial effect obtained through treatment with hypophyseal preparations. Beyermann's first two arguments are of questionable value. In view of the general unreliability of commercial preparations of the hypophysis designed for oral administration, and the failure to duplicate Beyermann's results, some doubt must be expressed in regard to the validity of the third argument. It is also possible that even in the presence of abnormally functioning gland, pituitary substance might exert some beneficial influence in cases of narcolepsy.

The beneficial effect of treatment with ephedrine sulphate might raise the question of deficient function of the suprarenal glands, although somnolence does not appear to be a characteristic symptom of Addison's disease (208). Although approving of Redlich's and von Economo's views, Bolten expressed the opinion that a disturbance of the vegetative centers in the floor of the fourth ventricle, particularly those regulating the chromaffin system, must also be considered. Involvement of these centers by an inflammatory process in the postencephalitic cases might lead, he reasoned, to diminished secretion of epinephrine. Bolten also considered the possibility of the infection having some adverse influence on the suprarenal glands themselves. In order to account for the cases of "genuine" narcolepsy, he postulated a slowing of metabolism as the result of congenital inferiority of the sympathetic nervous system and the glands connected with it. Dysfunction of the thyroid gland as a part of some polyglandular disturbance was considered a possible factor by Wenderowic, Adie, Redlich, and Bolten. The benefit obtained from medication with thyroid gland in a few cases does not furnish a very strong argument, however, for primary hypofunction of the gland. It is possible, of course, that some disturbance of the centers controlling the endocrine system as a whole may lead indirectly to altered function of any one of the individual glands.

Wagner expressed the belief that the cataplectic attacks were entirely different from the attacks of sleep, and that they were attributable to disturbance of the calcium-potassium balance originating in the floor of the third ventricle. This conjecture was supported, he believed, by his experiences with injections of afenil which in one of his cases precipitated a cataplectic attack. Cataplexy, however, cannot be divorced entirely from the attacks of sleep, and Wagner's observations might be interpreted in other ways, since injections of afenil remained without effect in his other case. Audo-Gianotti assumed on the basis of Demole's work that a decrease of circulating calcium and its accumulation in the infundibulum induced sleep. According to Kleitman, however, it has not been conclusively proved that the amount of circulating calcium is lowered during sleep. Although sleep has been produced experimentally by the injection of weak solutions of calcium into the tuber cinereum, proof is lacking that calcium normally accumulates in that region during sleep. Audo-Gianotti also expressed the view that disturbance of the normal cycles of sleep and waking, anabolism and catabolism, vagotonia and sympathicotonia, would account for the narcoleptic attacks. The narcoleptic attack, like normal sleep, is often assumed to represent a vagotonic state, but this is not strictly true. As Janota has pointed out, certain features of the two, for example, the diminished secretions of the mucous and lacrimal glands, are more suggestive of sympathicotonia.

Adie (1) and Cave compared the attacks of sleep observed in narcolepsy to the tendency of Pavlov's dogs to fall asleep during his work on conditioned reflexes. In both instances, as Adie and Cave brought out, monotonous stimuli seemed peculiarly liable to induce sleep, and extraneous stimuli to prevent it; cataplexy could be explained on the basis of partial inhibition. Adie saw a further analogy in Pavlov's statement that lively animals with a more labile nervous system were much more inclined to fall asleep under the conditions of his experiments than were quiet animals. Adie wrote, "It seems then, as if narcolepsy is an expression of fatigue in individuals with a kind of nervous activity that favors the spread of inhibitions and allows excessive emotional responses, further that the local response to inhibitions wherever they arise is abnormal, and that the symptoms are due to a general alteration of nervous activity rather than to abnormal stimuli which affect normal structures at a distance." Adie concluded, however, that the inhibition arose primarily from the subcortical centers. Rosenthal (202), in commenting on Pavlov's work and Adie's interpretation of it, expressed a preference for the word "dissociation," a term used by Trömner in his consideration of sleep. The tendency of one of the patients seen in the clinic to pass into an amnesic state in two particular situations, it might be remarked, was rather suggestive of a conditioned reflex.

Partial inhibition of cortical function would account very well for the attacks of sleep, the states of diurnal somnambulism, and, since the higher levels are involved in the fusion of visual images, also the transient diplopia. The diminution of muscle tonus and other bodily changes characteristic of sleep, however, would suggest that, in the attacks of sleep at least, there is a spread of inhibition to the basal nuclei and even lower centers. Since sleep represents a response to fundamental organic needs, furthermore, one would expect the inhibitory process to originate, under ordinary conditions, at subcortical levels. The cataplectic seizure also suggests a type of response integrated at lower levels, even though the exciting stimulus may enter these levels, in many instances, by way of the cortex. Cobb has suggested that the assumed diencephalic localization of the disease is probably common but not essential for the complete narcoleptic syndrome, just as localization in the rolandic area is common but not essential for the motor fit.

Brains of narcoleptic patients have yet to be studied, but I would venture to predict that when such studies are made, provided current methods of exploration are used, the findings will be meager, for the most part, and even more difficult of interpretation than is the case with epilepsy. Rosenthal has recently expressed a similar view; this opinion, like mine, was based largely on the impression that the symptoms of narcolepsy represent little more than gross exaggerations of normal weaknesses of the flesh. Diurnal drowsiness is obviously a common failing, and many of us are aware of relaxation of certain parts or experience difficulty in bringing certain muscles into play under conditions of emotional stress. Paskind's recent work on the effect of laughter on muscle tonus is interesting in this respect.

SUMMARY AND CONCLUSIONS

In a study of 377 cases of narcolepsy, including 147 observed at The Mayo Clinic, the findings of other observers relative to the frequency of onset in adolescence and early adult life were confirmed. In a larger group of 439 cases, males outnumbered females by a proportion of roughly 2:1. Numerous etiologic factors, including heredity, preceding infection and injuries to the head were considered. It did not seem reasonable to assume that all post-infectious cases were necessarily encephalitic. Besides cases in which the symptoms seemed definitely attributable to epidemic encephalitis or to trauma, others were considered in which the relative importance of infection or injury was difficult to evaluate. Aside from the signs and symptoms characteristic of the underlying condition, and perhaps the relatively better prognosis, it was found that symptomatic narcolepsy might differ in no way from the idiopathic form. The possible existence of constitutional factors in cases belonging to both groups could not be denied. It finally became apparent in the course of the study that the essential anatomic or functional changes might owe their origin to a variety of factors.

Aside from the two principal symptoms, attacks of sleep and cataplexy, attacks presenting various features of both were described. Attacks of powerlessness occurring as the patient was about to fall asleep, or as he awakened, and associated at times with dreams and hallucinations, were considered in detail. Attacks of transient diplopia and other ocular phenomena, hypnagogic hallucinations, and peculiar amnesic states were described. All these manifestations of narcolepsy appeared to be interrelated. The hypothesis that all represented states of partial inhibition, similar if not identical with the form of inhibition called sleep, seemed supported by clinical observation. Disturbances of nocturnal sleep appeared to occur with sufficient regularity to warrant the conclusion that the sleep regulating function of a narcoleptic patient may be profoundly disturbed. The tendency to gain weight early in the course of narcolepsy was fairly common. In a few cases, the malady seemed to have favored the process of growth. Disturbances of the sexual function, although not common, were considered to be of some significance. Aside from a certain degree of irritability likely to be manifested by any drowsy person, changes of personality were not common among the cases studied. The manner in which patients faced the problems presented by their infirmity indicated that they were of a stable nature. In only two cases, including one studied at the clinic, narcolepsy seemed to have led to the development of a rather characteristic psychosis. Extensive changes had occurred in the emotional life of another patient. Eighty patients previously examined in the clinic were traced. Although not inconsistent with a long and healthy life, the malady pursued an essentially chronic course; considerable amelioration in the severity of the symptoms was not common.

The results of clinical examination in cases of narcolepsy were presented in some detail. The characteristic sleepy, but healthy and well-nourished appearance of the patient was commented on. Slightly more than half of the patients examined in the clinic were overweight. Aside from occasional polyuria of mild degree, no particular disturbance of the water balance was noted. Apart from a certain tendency to plethora, examination of the blood revealed nothing in particular. The tendency to lymphocytosis, so pronounced among Europeans, was not evident among patients examined at the clinic. The values for blood sugar in several cases lay near the lower limit of normal, but study of the sugar tolerance in twelve cases showed no constant deviation from normal. Although the basal metabolic rates varied markedly, low rates were more common than high rates, this being especially true among the women. Anomalies of the sella turcica were so uncommon among the patients examined at the clinic that considerable doubt was expressed as to the significance of the finding.

Following a rather extensive consideration of post-encephalitic and post-traumatic narcolepsy, the possible associations of somnolence with other organic diseases of the brain or surrounding parts, pregnancy, diabetes mellitus, obesity, polycythemia vera, deficient oxygenation of the blood, malnutrition, and feeblemindedness were reviewed. After a brief review of certain states clinically related to narcolepsy, the possible relationships between narcolepsy and epilepsy were considered. The various methods of treatment were reviewed, the symptomatic relief afforded by the administration of ephedrine sulphate being considered in some detail. There is a brief summary of the views that have been expressed relative to the nature of narcolepsy. The assumption, based entirely on reasoning by analogy, that the malady was attributable to a disturbance in the floor of the third ventricle, seemed most likely.

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THE VITAMINS AND RESISTANCE TO INFECTION

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CONTENTS

I. Introduction 123
II. Vitamins A and D 124
III. Vitamin A 145
IV. Vitamin D 157
V. Vitamin B 175
VI. Vitamin C 190
VII. Vitamin E 206

INTRODUCTION

In many of the investigtions on the relation between the vitamins and resistance to infection rations lacking in several essentials have been employed, usually in an effort to test the effect of inadequate human dietaries. Although such investigations have yielded results of practical value, they do not disclose the rôles played by the diverse missing substances. More definite information on this question can be obtained from experiments in which diets deficient in one vitamin only are utilized and the following review has been limited, with very few exceptions, to the discussion of such work. Very numerous papers on this subject have appeared and some no doubt have been overlooked by the author. Wherever possible the investigations have been described in sufficient detail for the reader critically to appraise them. Unfortunately many of the experiments have been carried out on such small numbers of animals that the results are not statistically significant.

The problem of whether the metabolic changes resulting from the deficiency of a vitamin are accompanied by changes in the defense mechanism has been attacked by at least four different methods, as follows:

(1) By the determination of any changes in the natural immune bodies or cellular reactions, due to the deficiency.

(2) By the assay of antibody responses following the injection of appropriate antigens into deficient and normal animals.

(3) By contrasting the liability of the adequately and defectively fed individual to spontaneous infections.

(4) By the comparison of the susceptibility of individuals on complete and inadequate diets to experimentally induced infections.

In addition some work has been published on the effect of the administration of added vitamins in various experimental and clinical infections. This constitutes section (5).

Each of the vitamins or vitamin complexes, including "fat soluble A" as one of the latter, will be discussed under these five sections.

Wherever possible each section has been further subdivided into subsections in which (*a*) the results indicating decreased resistance and (*b*) the results indicating no decreased resistance are collected together. Some apology should perhaps be offered for this rather unusual arrangement of the material, but it has the advantage of added clearness and it causes little interference in the sequence of thought as very few of the investigations are based on the work of others.

Short summaries are found at the end of many of the sections, especially of those in which the reports have yielded conflicting results.

VITAMINS A AND D

In the early work on the vitamins, the substance known as "fat soluble A" was thought to consist of one factor only. It was later found to include both vitamin A and vitamin D. Both these factors have been deficient in the diets used in many experiments and therefore a section has been included in which the effect of this dual deficiency is discussed.

1. Variations in the natural immune bodies or tissue reactions due to vitamins A and D

(*a*) *Results indicating that these are reduced in vitamin A and D deficiency.* The phagocytic index when determined in vivo was reported by Werkman (1) to be slightly lower in rats and rabbits lacking these vitamins than in controls fed adequate diets. Findlay and MacLean (2) in addition found a reduction in the bactericidal action of staphylococci of the whole blood of such malnourished animals after the onset of xerophthalmia or other epithelial infections. However, as such a reduction accompanies all acute infections, vitamin A can not on this evidence be held responsible for it. In normal rabbits an antibacterial substance known as lysozyme (Fleming) is present in the tears. However when these animals are fed vitamin A and D deficient diets this substance disappears from these secretions (Findlay (3)).

(*b*) *Results indicating that these are not reduced in vitamin A and D deficiency.* The complement titre was found by Cramer and Kingsbury (4) to be the same whether the rats were fed diets that were normal or were lacking in these vitamins. When the phagocytic index was determined in vitro no difference was found between rats on the deficient or the complete diets (Werkman (1)), and Findlay and MacKenzie (5) were not able to demonstrate any defect in the opsonic power of the inadequately fed animals after 10 weeks on the diet, when only one of the four animals, whose sera were pooled for the test, had developed xerophthalmia.

2. Variations in acquired immune bodies or tissue reactions due to vitamins A and D

(*a*) *Results indicating that these are reduced in vitamin A and D deficiency.* In 1928, Blackberg (6) reported the following findings using vitamin A and D deficient and normal rats. If dead typhoid bacilli were injected the deficiently fed animals produced considerably smaller amounts of agglutinins and bacteriolysins than the controls. When, however, living bacilli were used the difference was very much less marked, and if larger amounts of the live culture were injected the difference was further lessened. The explanation given was that the large doses of typhoid bacilli probably contained substances which partly compensated for the inadequacies in the diet.

Lassen (7) states that he has demonstrated a decreased production of agglutinins after intraperitoneal injections of the Breslau paratyphoid strain in vitamin A and D deficient rats as compared with normal rats. However, only seven rats were used in the tests and the differences were slight and probably negligible. When the agglutinin titre was compared in rats fed these diets, which had been immunized and later infected per os with the same strain, the differences were more marked, but as the oral infecting dose was proportional to the weight of the animal the normal rats received larger doses, and in addition these normal rats developed less severe infections from which they recovered more rapidly. It seems doubtful, therefore, whether the animals used in these latter tests were comparable.

(*b*) *Results indicating that these are not reduced in vitamin A and D deficiency.* At least three reports (4a, 8, 9) have shown that there is no reduction in the agglutinin or bacteriolysin production which follows the injection of typhoid bacilli into rats or rabbits red vitamin A and D deficient diets. Werkman (9) could also find no reduction in the precipitins to human sera or the hemolysins to sheep's red blood cells in his deficiently fed rats and rabbits. It has also been reported that phagocytic indices of immunized animals, whether vitamin A and D deficient or normal, are equally high (1).

Summary of immunological investigations. Except for the findings of Blackberg's first experiments, which were quite the reverse of those already reported (Zilva, Werkman), and for Findlay's statement that lysozyme disappears from the tears, no evidence has been brought forward to show that the immune reactions of these deficient animals are inferior to those of adequately fed controls. The lachrymal gland does not function normally in these deficient animals, and it is not surprising that the lysozyme in the tears is reduced. The significance of the slightly reduced phagocytic index when determined in vivo, which Werkman found in the non-immune deficient animals is practically nullified by the fact that this difference could not be shown when in vitro methods or immune animals were used.

3. *Occurrence of spontaneous infections in vitamin A and D deficiency*

(*a*) *Infections indicating a reduced resistance. I. Experimental.* A great many papers have been published on the occurrence of natural infections in vitamin A and D deficient rats and the results of some of the more important investigations will be outlined below.

In 1913, Osborne and Mendel (10) described an abnormal eye condition, later known as xerophthalmia, which appeared in rats that were kept on a vitamin A and D (i.e., fat soluble A) deficient diet. A few years later, several authors (11) stated that respiratory infections were also frequent in such animals, but some of the more recent work (12) has shown that such lesions are relatively rare. Sherman (13) however, found that the vitamin A content of the rat's lung varied with the vitamin A concentration in the diet. In 1923, Daniels (14) noticed when examining the heads of rats that had been fed a deficient diet for about 10 weeks, that the nasal sinuses and middle ears were always filled with pus and that some of the animals showed in addition abscesses at the base of the tongue and lung infections. Later investigations (12a, 15) demonstrated that rats kept on vitamin A and D deficient rations often developed infections in the nasal sinuses, middle ear, eyes, salivary glands, lungs, kidneys, bladder, pancreas, uterus, epididymis and other genital organs. These infections apparently always originated in the proximity of epithelial tissue and were all of a subacute nature. The bacteria isolated from such lesions in the upper respiratory tract were similar to those found in these locations in normal rats, although some species tended to predominate (16). It is likely therefore that the bacteria which set up these infections were some of those naturally present in the locality, although Mellanby (17) claimed that in at least some cases (vitamin A deficient only) the infection was blood borne. The situation was very much clarified by Wolbach and Howe in 1925 (12a), who found that in vitamin A and D deficient animals the columnar epithelium which normally covers the nasal mucosa, trachea, etc. was replaced by keratinizing, stratified epithelium. The process apparently starts as a number of small foci of proliferating squamous epithelium which spread out and undermine the columnar epithelium, which is later desquamated. frequently in sheets. The foci finally coalesce and the original columnar secreting epithelium is completely replaced by glandless and therefore to some degree non-functional squamous epithelium. Even in the rat, the order and frequency with which the organs are affected varies considerably in different laboratories (18) and variations also occur in the different species of animals (e.g., the rat, the monkey (19), man (20), etc.).

The part played by bacteria in the production of these lesions has been the subject of numerous investigations. Several authors (12a, 21) came to the conclusion that the metaplastic changes preceded infection which did not necessarily occur at all. Others (18a, 22) affirmed that infections might exist prior to the epithelial alterations.

The general consensus of opinion at present is that metaplasia plays the primary rôle and that infection is a very common secondary complication. The susceptibility of this metaplastic epithelium to infection may be due to several factors such as:

(1) The absence or reduction of the mucous membrane secretions which not only wash off bacteria and other particles but also have an active bactericidal action.

(2) The presence of epithelial débris, particularly in glands whose ducts have been blocked, provides a favorable medium for the growth of bacteria that are already present.

(3) The possibly increased permeability of the metaplastic or the intestinal mucous membrane.

(4) Artificially induced infections in such animals (which will be described in the next section) suggest that the general as well as the local resistance of these animals may be lower than normal.

It has been quite definitely proven that the rapidity with which xerophthalmia or these other lesions develop varies with the age (13) of the animal, and their previous diet (23). However, in addition to these predictable variations, other very marked differences in susceptibility occur (24) even in litter-mates kept under apparently identical conditions. Whether this means that individuals, possibly humans as well as animals, vary in their requirements for this factor or merely in their ability to absorb it from their food is at present a matter for speculation.

Slight abnormalities in the mucous membrane can be detected long before any external signs of vitamin A deficiency appear. For instance, by means of a corneal microscope and a slit lamp Mouriquand (25) revealed microscopic ulcers of the cornea about 20 days before the onset of xerophthalmia. In addition Thatcher and Sure (26) discovered metaplasia of the posterior part of the tongue and of the respiratory tract some time before any other indication of the deficiency was evident. These findings suggest that slight degrees of deficiency may have a deleterious effect on the animal.

There is a lack of agreement on the question of whether infectious lesions in the substance of the kidney occur in such animals. Wolbach and Howe (12a) state that these occur very rarely, whereas several other investigators (24, 27) frequently found nephritis in their animals.

A deficiency of vitamin A is also associated with the development of kidney and bladder stones (28).

One often sees it stated that lesions in the alimentary tract are frequent in vitamin A and D deficient animals. This statement is somewhat misleading for although the salivary glands are very commonly involved, the rest of the gastro-intestinal tract, except for a very slight atrophy of Brunner's glands (12a), escapes these metaplastic changes according to Wolbach and Howe. However, Cramer (4) and others state that the intestinal wall is thin, and Seidmon and Arnold (29), Lassen (30) and others (4) have demonstrated that it is more permeable to bacteria. Cramer (4,b) in addition found atrophy and degeneration of the intestinal villi and also increased numbers of intestinal protozoa and bacteria between the villi and in the glands. McCarrison (31) claims that vitamin A, as well as vitamins B and C, plays a part in maintaining the protective power of the intestinal mucous membrane against invasion.

May Mellanby (32) found that the sulci between the teeth and the gums became deeper and wider and lined with abnormal epithelium in dogs fed these deficient diets during early life. When these sulci later became infected, pyorrhea was the result. Shibata (33) reported that when rats were fed a similar diet, they also developed this disease. According to Kobashi (34), rats which lack these vitamins are more seriously affected by a mite (Notoedres alepis) which burrows into the skin of the ears and tail than are normal animals.

If cod liver oil or raw cabbage, both of which contain much vitamin A, or carotene, which is converted into vitamin A after ingestion, is fed to an animal that is showing early signs of vitamin A deficiency, recovery may be complete. The normal columnar epithelium grows again, the infections clear up and apart from possibly some slight scarring (18), no evidence is left of the lesions. However, if the lesions have become severe before remedial measures are adopted, complete recovery is not possible (14). The addition of vitamins A and D to a diet that would otherwise lead to the formation of urinary stones will prevent their appearance (28b), and the same holds true for the other manifestations of this deficiency.

II. Clinical. Large series of cases of human xerophthalmia have been reported from Brazil, Russia, Japan, Denmark, and China (36), and this condition occurs occasionally on this continent (20, 37). Spence (38) discovered seventeen cases of xerophthalmia and night blindness, which is a symptom of vitamin A deficiency in man, during one year in a large city clinic in England. The patients were mostly older children, and, although their growth was about normal, two-thirds of them suffered from persistent skin infections which cleared up rapidly, as did also the eye symptoms, when an adequate diet including cod liver oil was fed. In 1917, Bloch (39) reported that children living on diets markedly lacking in vitamin A were very susceptible to respiratory and genito-urinary infections for some time before they developed xerophthalmia. This same author stated some years later that 80 per cent of the Danish infants that were admitted to hospitals suffering from xerophthalmia showed severe infections elsewhere, e.g., pneumonia, bronchitis, otitis media, pyelitis and pyodermia, and that barely two-thirds of them survived to the age of 8 years, which would suggest that they were left in a state very susceptible to infection. He also reported that there was a great deal of sickness among the older children of Central Europe during the war, when the dairy produce was rationed so that the young children and the nursing mothers obtained a certain daily amount. He concluded that the ill-health of the older children was due to dietary deficiencies (probably of vitamins A and D). Several others (40) have observed that the incidence of infections was high in groups of xerophthalmic children.

During an epidemic of grippe, Niemann and Foth (41) observed that three out of twelve infants fed "buttermeal" (Buttermahl) or other diets which were rich in fat and vitamin A died, whereas nine out of eleven infants on a carbohydrate-rich vitamin-poor ration succumbed.

An epidemic of so-called distorted pneumonia associated with a non-contagious granular conjunctivitis broke out in an industrial school near Edinburgh, and Findlay (42), who was called in, recognized the condition as early xerophthalmia. It was discovered that the food, which was low in vitamins A and D, was cooked for several hours in sealed cauldrons, and when cod liver oil and raw swede juice were given. along with a properly cooked diet, the epidemic cleared up rapidly.

Cody (43) reported that a diet deficient in these vitamins may give rise to a chronic, non-foetid rhinitis, and he found that the generous feeding of these factors was helpful in its treatment. In addition, he claimed that cod liver oil (vitamins A and D) was of great value in the prevention of infections in the upper respiratory tract and ears, and that hypertrophied tonsils were less frequently seen when it was used.

Dean (44) stated that in general babies receiving low fat (1 to 1.2 per cent) diets more frequently show mastoid infection than those fed 2.5 to 3.0 per cent fat.

A hitherto undescribed dermatosis, associated with the clinical manifestation of vitamin A deficiency in the majority of cases, was reported by Loewenthal (45) from East Africa in 1933. Papules were formed from altered hair follicles and these were very irritable, and were surrounded by considerable perifolliculitis. The treatment was confined to one ounce of cod liver oil daily and this cured 98.6 per cent of the cases in nine weeks time.

After examining the postmortem reports on twelve infants that showed evidences of vitamin A deficiency, Wolbach and Howe (18b) concluded that the maximum effect was on the respiratory tract and that the eyes were affected in only the most extreme cases. In other words, the absence of xerophthalmia does not rule out the possibility of vitamin A deficiency.

On studying many of these reports one is impressed by the great variations in the effects produced in individuals by the same degree of shortage of these vitamins. McCarrison (46) is of the opinion that more of these factors are required under certain conditions, such as during reproduction, including lactation, during debilitating diseases and acute infections, during periods of very heavy work (two of Spence's (38) cases were precipitated by the latter), during exposure to cold, and also in larger individuals. In addition, Bloch suggests, very plausibly, that the inability to properly utilize fats would increase the amount of these vitamins needed to maintain normal health. It has also been fairly well established (47) that more of one vitamin is needed when the food is very rich in another vitamin.

(*b*) *No increase in infections.* In contrast to above reports Spence (48) (1930) found that infections were neither more frequent nor more severe in seventeen patients with xerophthalmia.

Summary of clinical reports. With the exception of Spence's 1930 report, all the evidence goes to show that a marked deficiency of these vitamins is associated with numerous infections. That mild degrees of deficiency, such as might be found among the poorer members of society, result in a reduced resistance is possible, although it has not been definitely proved. The work of Spence (1931), Cody and Dean would suggest that such was the case.

4. *Susceptibility to artificially induced infections*

(*a*) *Reduced resistance in vitamin A and D deficient animals.* Werkman (49) compared the resistance of rats which had been fed diets lacking in these vitamins until signs of xerophthalmia appeared with that of others of the same weight fed complete diets. He found that a much larger percentage of the deficiently fed rats succumbed after the injection of typhoid or anthrax bacilli or pneumococci. The resistance of rabbits to infection with B. anthracis was similarly lowered when vitamins A and D were withheld. He later reported that a much smaller amount of diphtheria toxin per gram of rat was needed to kill a vitamin A and D deficient rat.

Groups of mice fed diets deficient in these vitamins and also normal diets were injected with varying amounts of mouse typhoid bacilli intraperitoneally by Hotta (50). A larger percentage of the former animals died after such treatment.

Three reports (7, 29, 30, 51) have been published which have shown that after feeding vitamin A and D deficient and normal rats paratyphoid cultures, these organisms can be recovered from more organs and in larger numbers from the deficient animals. Seidmon and Arnold (30) kept their full-grown animals on the diets for about 10 weeks before they infected them and the organs were cultured one-half hour and one hour after infection. Far more of the organs from the inadequately fed animals yielded the bacteria, which according to these authors indicates greater intestinal permeability. Verder's (51) rats, which were also full-grown, were kept first on the deficient, then on an adequate, and finally on the deficient diet for about six weeks. Six of these rats with a similar number that had been kept on an adequate diet throughout were killed and examined one and four days after an oral infection with B. enteritidis. The only significant finding was that half of the deficient rats showed positive spleen cultures. Two of the control rats which had refused to eat any food containing cod liver oil for the last seventeen weeks of the experiment also showed positive spleen cultures.

Lassen (7, 30) has reported the results of a large series of experiments in which he fed Breslau (Aertrycke) strains of paratyphoid bacilli to normal and vitamin A and D deficient rats and then cultured bacteriologically many of the organs and glands. The normal animals were slightly vitamin D deficient, as 15 per cent of butter fat was the only source of this factor. The controls and the deficiently fed rats were infected when the latter began to show "more or less xerophthalmic changes," which was usually after 5 to 7 weeks on the diet. In a typical experiment most of the deficient rats died about fourteen days after infection and the organism was found widespread throughout the body, and often in the blood stream. Controls killed about this time would usually yield positive cultures from only the mesenteric glands and Peyer's patches. If the controls were allowed to live two weeks longer, all the cultures were sterile. The few deficient animals alive at this time were found to still harbor the organism in some of their glands and organs. It is unfortunate that no uninfected diet controls were included, as deaths from the avitaminosis alone may occur after similar intervals. An even more striking difference in resistance in favor of the adequately fed rats was shown when the culture was injected subcutaneously. This author is of the opinion that the resistance is slightly lowered after 2 to 3½ weeks on the diet, but thinks that a very marked decrease occurs coincident with the onset of xerophthalmia.

Hiraishi (52) fed a diet lacking in these vitamins to young pigs and succeeded in infecting them with human and pig ascarid worms which, according to Nagoya (53), had up to that time been found to be impossible. Nagoya (53) himself infected a series of puppies, some of which were on vitamin A and D deficient diets and some of which were given complete diets, with a dog hookworm. He found that a larger percentage of larvae were recovered from the body, and more of them reached maturity, when the pups were fed the deficient ration.

Kobashi (54) found the "morbidity was greater" and the symptoms more severe from a purulent submaxillary adenitis, set up by feeding a coccobacillus isolated from a similar spontaneous adenitis, in vitamin A and D deficient rats than in adequately fed controls. The abscesses which followed the injection of staphylococci into the skin also ran a much more severe course in such deficient animals.

Tuberculosis. When bovine tubercle bacilli were injected into the peritoneums of rats lacking these vitamins, omental tumors which contained these organisms were produced. The same treatment in normal animals did not provoke this reaction (Cramer and Kingsbury (4)).

Hagedorn (55) used a similar infection in his experiments and he reported that whereas 54 per cent of his normal rats lived, only 25 per cent of the deficient rats survived. In addition, the latter showed earlier generalization and more widespread lesions.

Smith and Hendrick (56) injected tubercle bacilli intraperitoneally into groups of rats on various diets. The control rats, which were fed a diet slightly lacking in vitamin D and of low biological value, developed serious clinical symptoms in 30 to 60 days, and died after 10 to 15 months. The diet of the group deficient in vitamins A and D was also of low biological value and these animals showed serious symptoms earlier and died in three or four months.

The basal diet (vitamin A and D free) which Gloyne and Page (57) used was so inadequate that young rats made practically no growth on it and died in about 43 days. If they were infected intraperitoneally with tubercle bacilli after two weeks on the diet, they died even sooner, on the average in 33 days. The addition of cod liver oil or whole milk to this ration caused not only much improved growth, but also enabled the rats to withstand the infection better. They were killed and examined after 39 and 57 days. No tuberculous lesions were found in any of the rats, but the bacilli were demonstrated in the spleen and glands.

Smith and Hendrick (56) also found that vitamin A and D deficient rats, which had been infected with tuberculosis frequently died after the intraperitoneal injection of large amounts of old tuberculin whereas similarly treated animals fed normal diets survived. Schütze and Zilva (58) did not find the same to hold true when they used smaller doses of tuberculin. However, in later experiments they increased the amount of tuberculin and were able to confirm the work of Smith and Hendrick. They showed however that the deaths were not due to a specific sensitivity to the tuberculin, as injections of Salmonella toxin into tuberculous deficient animals killed about the same percentage of them.

No reduced resistance in vitamin A and D deficient rats. L. B. Lange's (59) experiments gave essentially negative results. She placed adult rats on various diets, one of which contained very little of the fat soluble vitamins (2 per cent of butter once a week). About a month later, she infected many of them intraperitoneally with bovine tubercle bacilli, and then killed them at intervals. Tuberculous lesions were practically absent beyond the regional lymph glands, and no differences of any consequence were found between the rats on the various diets. She concluded that although the rat was suitable for nutrition experiments it was unfortunately very unsusceptible to tuberculosis.

In 1926, Grant (60) reported that the intestinal walls of guinea pigs that were given very large daily doses of cod liver oil (3 cc.) were more permeable to B. aertrycke after oral infection than those of guinea pigs without this oil. The permeability was determined by culturing the organs and the blood. Only two controls were used, and six were fed the cod liver oil containing diet.

In one experiment only Eidinow (61) fed six rabbits 1 cc. of radiostoleum (vitamins A and D) daily for twelve days and then infected them and also two untreated controls with staphylococci intravenously. The animals all died within twenty-four hours. This author had previously found that 4 out of 30 rabbits that were exposed to a "daylight" lamp for 48 hours before this infection survived. In view of this very low percentage of survivors, the number of animals in the radiostoleum experiment was too small.

In 1932, Nègre (62) stated that if after a subcutaneous infection with B. tuberculosis 1 cc. of cod liver oil was fed to a guinea pig daily, the tuberculous involvement of the spleen and glands was greater than if this oil was omitted.

Summary of artificial infections. Several criticisms apply to the majority of these studies, for example:

(1) With the exception of Lassen's work, the conclusions were based on results obtained with very small numbers of animals.

(2) Non-infected diet controls were not followed except by Lange. As this avitaminosis can cause death, such controls should be included.

(3) The diets lacked vitamin D as well as vitamin A. The antirachitic effect of vitamin D is so striking that its other rôles in nutri-

tion have been frequently overlooked. It does however quite definitely promote growth, is a factor in the prevention of dental caries, and in addition is of importance in maintaining a high resistance to infection (see section on Vitamin D).

(4) In the majority of instances, the animals were kept on the deficient diets for a considerable time before they were infected. Most of them were probably suffering from spontaneous infections and were in poor condition before they were artificially inoculated. It is not surprising that the added burden of another bacterial invasion was not borne by them as well as by the healthy animals. Lassen did infect rats after short periods on the defective diet, but this might well be done again, and a better method of measuring the resistance might be employed.

(5) The infection was in a good many instances established by procedures far removed from the natural method of infection. The use of more natural methods is probably preferable.

The work which did not demonstrate any decrease in resistance can be dismissed rather briefly. No definite infection was set up in Lange's experiments, and the amount of cod liver oil and radiostoleum that Grand and Eidinow gave was excessive. Nègre's results can not be explained away on these grounds, but his experiments were carried out on very few animals.

The great mass of evidence therefore suggests that a considerable reduction in resistance to infection occurs in animals suffering from a marked and prolonged deficiency of the fat soluble vitamins.

5. *The use of vitamins A and D in infections*

I. Experimental. Smith (63) found that guinea pigs maintained on a normal diet lived longer after intraperitoneal infection with tubercle bacilli than others on the same diet reinforced with 0.5 per cent of cod liver oil. However, fewer of the latter animals showed extensive generalization.

Schütze and Zilva (58) on the other hand suggested that rats which were fed just enough vitamins A and D for normal growth (as cod liver oil) developed larger omental tumors after the injection of B. tuberculosis intraperitoneally than similar animals whose diet contained a superabundance of cod liver oil.

II. Clinical. (*a*) *Results indicating an increased resistance.* *1. Prophylactic effect.* During a period of 4½ months Widmark and Svennson (64) compared the height, weight, absenteeism, etc., of primary school children with and without the addition of cod liver oil (15 grams weekly) to their regular diet. There was no difference in the height and weight increments, except in the 14-year-olds, where those fed the cod liver oil showed some increase in weight and a considerable increase in height. The girls, whose home conditions were somewhat better than those of the boys, showed no difference in absenteeism. The boys showed slightly less time missed from school (3.8 days) when given the cod liver oil, and fewer days missed per sick child (6.0 days). The control figures were 4.4 and 7.0 days respectively. This difference might suggest that the boys' diets were partially deficient in vitamins A and D.

In 1931, Mellanby and Green, with the assistance of Pindar and Davis (65), tried out the prophylactic effect of these vitamins in pregnancy. They fed a series of 275 pregnant women concentrates of vitamins A and D (radiostoleum, ½ drachm daily) usually for four weeks before term. The morbidity, according to the B. M. A. standard (which means a fever of over 100°F. from the first to the eighth day postpartum), of these women during the puerperium was 1.1 per cent, whereas 4.7 per cent was the figure in a corresponding number of control cases that were delivered during the same period. When the cases were also compared on the basis of the duration of pyrexia, the beneficial effect of the vitamin supplement was again evident.

In the next year, eighty-six patients were similarly treated in Glasgow, and it was reported (66) that "on the whole, it was concluded that although vitamin A may gradually increase the general resistance and the organismal infection may thus be controlled, this does not reach a degree sufficient to deter any but the least virulent types of sepsis, in which a similar effect could be obtained by less specific measures."

Cameron (67) has also reported beneficial results in a smaller series of pregnant women who were advised as to their diets and given in addition adexolin, which is a concentrated source of vitamins A and D. Fifty-seven untreated cases showed a morbidity of 8.7 per cent compared with a 5 per cent morbidity among 59 cases treated with adexolin. In a large proportion of all these morbid cases the infections were probably in the uterine mucosa and walls.

Holmes and his co-workers (68) gave 185 industrial workers (various types of work) a tablespoon of cod liver oil daily and compared their respiratory infections and time off with that of a similar group of 128 persons that received no treatment. Fifty-five per cent of the cod liver oil group had no colds during the four winter months of the experiment and only 33 per cent of the controls showed this freedom. Fifty-two per cent of the cod liver oil group and 41 per cent of the controls lost no time from their work during the four months. The diets of these individuals may have been so low in vitamins A and D that their respiratory mucous membrane was not functioning normally and the cod liver oil may have cured the incipient metaplasia.

2. Treatment of infections. Acting on the supposition that the vitamin A supplies of the mother are reduced by the end of the pregnancy and that she may therefore be more susceptible to infection, Mellanby and Green (69) treated three cases of puerperal septicemia with a concentrate of vitamins A and D (radiostoleum, 4 drachms daily). All three recovered, although they had had positive blood cultures. The beneficial effect of the treatment was not evident for one to two weeks and one of the cases received quinine as well. In the previous two years only two out of twenty-four such cases (8 per cent) recovered. Although the results with the vitamin treatment were very encouraging, they should not be accepted without question, as the severity of these infections often varies markedly from year to year, and in some years they are distinctly less fulminating than in others.

Hattori and Matsuura (70), working in the Nagoya Sanitorium, studied the clinical effects of cod liver oil and vitamin A, and concluded that vitamin A was very useful in the treatment of tuberculosis. They state however that chemically pure preparations of vitamin A are not as effective as cruder preparations.

In 1929, Dean (71) found that diets containing generous amounts of vitamins A and D speeded up convalescence after drainage operations for chronic nasal sinusitis in children.

Holmes and Ackerman (72) gave a series of 28 underweight children which could all be classed as tuberculosis suspects (contacts, arrested gland cases or cases with daily fever) two teaspoonsful of cod liver oil per day for about six months. Seventeen of them gained about twice as much, and the others gained considerably more, than the expected amount. All but two showed reduced temperatures and the attendance records were much better than in the preceding year.

Whether the course of pneumonia could be benefited by the administration of a concentrate of vitamins A and D (radiostoleum) was investigated by Donaldson and Tasker (73). The patients were all South African native mine laborers. One hundred of these were given the usual routine treatment, including in a few cases convalescent serum and diathermy, and forty-two were given four teaspoonsful of radiostoleum daily. The cases were placed in the groups on admission without selection. Thirteen per cent of the cases given the routine treatment died, and only 7 per cent of the vitamin treated cases succumbed. None of the deaths occurred within one day after admission and 90 per cent of them took place five days or more after entrance into the hospital. The native diet was probably deficient in vitamin A. The course of the disease was a little less severe in the vitamin treated cases, and none of these developed pyogenic infections, whereas two of the routine cases did so. These results suggest that such treatment is of value, but the repetition of the work on a larger series is necessary before final judgment can be passed.

Pattison (74) found that concentrates of vitamins A and D were no more valuable in the treatment of bone tuberculosis than cod liver oil.

In the next year (1931), Flamini (75) reported that the combination of vitamins A and D in large doses had a frankly beneficial effect on the course of infectious diseases in nursing children and in children recently weaned.

In treating cases of otitis media, Cody (43) found that the administration of large amounts of vitamins A and D shortened the duration of the discharge, accelerated the recovery of normal hearing and apparently rendered mastoid complications less frequent.

Ellison's (76) results in the treatment of measles, which is a disease that is particularly common among the poorer classes, were very encouraging. In measles the epithelial tissues of the respiratory tract are seriously injured and the skin is prone to secondary infections. Three hundred cases of measles under 5 years of age were treated with a cod liver oil concentrate (adexolin), equivalent to 1 ounce of cod liver oil daily, for from seven to twenty-one days, according to the severity of the case. A similar number of untreated cases during the same period served as controls. The mortality of the controls was 8.7 per cent and that of the treated cases was 3.7 per cent, and practically all of the deaths were due to pneumonia. Almost an equal number of cases of pneumonia occurred among the treated cases, but two-thirds of them recovered. The ear and skin complications were not benefited by this treatment.

Mundaliar and Menon (77) reported that prolonged puerperal pyrexia, of 6 to 26 days duration, quickly subsided after the administration of vitamins A and D. Only five cases in all were followed and other forms of treatment were given preceding that described. The results were very promising but need confirmation.

A cod liver oil concentrate was injected subcutaneously into tuberculous patients by Gordon and Titherington (78). About 13 per cent of them showed some gain in weight, and about half of those that were suffering from upper respiratory infections (sinus disease and bronchitis) were improved.

Several investigators (79) have shown that concentrates of vitamins A and D in addition to small amounts of iodine are often very satisfactory in the treatment of exophthalmic goitre.

(*b*) *Results indicating no change in resistance.* Barenberg, Greene and Abramson's (80) study of the incidence of pneumonia in institutionalized infants with and without cod liver oil will be discussed in detail in the section on Vitamin D, as the unprotected group showed rickets. They did not find however that the cod liver oil reduced the frequency of pneumonia.

Burton and Balmain (81) showed that about one-third of the Dick-positive pregnant patients to whom they gave large amounts of radiostoleum (vitamins A and D) became Dick-negative. However, the original positive reactions were slight and the change may have been due to the repeated tests with the toxin (Dick tests). It is almost impossible to imagine that the feeding of these vitamins would specifically increase the streptococcus antibody production.

The same authors also treated scarlet fever cases with the same vitamin concentrate, taking as their criterion the per cent of negative Dick tests on discharge. Sixty-five per cent of the treated cases and

60 per cent of the control cases showed such negative tests. This difference is probably negligible.

In 1931, Hallam (82) reported that chilblains were as prevalent among children on an average diet as among others that were given additional amounts of vitamins A and D.

As rheumatic fever is more common among the poorer classes whose diets are often low in the fat soluble vitamins, Warner (83) fed children, who had already had one attack of this disease, vitamins A and D (radiostoleum) and calcium salts, in the hope that this would reduce the frequency of rheumatic relapses. The treatment was found to be of no appreciable value. It is possible that the organs which are attacked by this disease are not affected by a deficiency of vitamin A.

Sutliff and his collaborators (84) administered 100 minims daily of a cod liver concentrate (the vitamin A was concentrated about 20 times) to over 300 scarlet fever patients for 10 days after admission, and recorded the incidence of otitis media. About 9.4 per cent developed this complication, whereas 11.3 per cent had developed it in the preceding year when no such treatment was given. The authors consider this difference to be of no significance. It is unfortunate that the control and treated cases were not followed simultaneously, as the severity of such diseases varies from year to year.

Summary of the use of these vitamins in infections. The value of these vitamins in prophylaxis against infections may be due to the fact that the diet of the individuals involved contained a suboptimal amount of these factors. The negative effect which Barenberg et al. reported may be explained by the assumption that the infants' diets already contained much vitamin A as milk.

The results of this treatment for puerperal infections are very striking but are based on very few cases, and it is surprising that confirmatory reports have not been published if such confirmation is possible. Puerperal sepsis is as high, or higher, on this continent as in Europe, and without doubt vitamin A is more abundant in the average diet here (85).

Respiratory infections seem on the whole to be benefited by these procedures, Ellison's work being particularly favorable. Aural complications in either measles or scarlet fever are however neither prevented nor helped. In contrast to this, Dean and also Cody found that the administration of these vitamins accelerated recovery after sinus or ear infections had set in.

The onset of an acute infection in an individual whose diet is low in these vitamins increases their need for these factors, with the result that some of the tissues probably become partially depleted. It would appear that the human lungs are particularly prone to be affected by these deficiencies (18b), and it is possible that diseases which affect the lungs secondarily would be well combatted by the use of vitamins A and D concentrates. From the results of these investigations one would infer that the tissues of the inner ear and joints are not much affected by the lack of these substances. Of the eighteen reports that have been reviewed, only two yielded entirely negative results. It would also appear that the administration of vitamin A along with vitamin D is possibly of more value than vitamin A alone (see section on Vitamin A).

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VITAMIN A

1. Variations in the natural immune bodies or tissue reactions due to vitamin A

Results indicating that these are not reduced in vitamin A deficiency. M. R. Green (1) has recently reported that the feeding of a vitamin A deficient diet to a rabbit does not reduce the natural hemolysin or complement titre of its blood.

2. Variations in the acquired immune bodies due to vitamin A

(*a*) *Results indicating that these are reduced in vitamin A deficiency.* Tanaka (2) found that in vitamin A and vitamin C free animals (rats and guinea pigs) the amount of agglutinins and amboceptors induced by the injection of cholera and typhoid bacilli was lower than in the controls.

When Green (1) injected typhoid bacilli into normal and vitamin A deficient rabbits and later determined the titre of anti-typhoid agglutinins and bacteriolysins, she found that the deficient animals produced slightly less of these antibodies. When sheep's red blood cells were used as the antigen the hemolysin production in the inadequately fed rabbits was very much lower than normal.

(*b*) *Results indicating that these are increased with added vitamin A.* According to H. von Euler (3), carotene (pro-vitamin A) "did not affect hemolysis in vitro nor react with amboceptor, but when it was given in excess to rabbits the amboceptor in their blood was increased."

3. Occurrence of Spontaneous infections in vitamin A deficiency

(*a*) *Infections indicating a reduced resistance.* The widespread areas of epithelial metaplasia and the frequent localized infections which Wolbach and Howe (4) described in vitamin A and D deficient rats, are found to the same extent in rats lacking vitamin A only, according to the work of Goldblatt and Benischek (5). The latter authors were of the opinion, however, that metaplasia and infection might appear simultaneously as well as consecutively. After a detailed histological study of similar deficient rats, Harris, Innes and Griffith (6) stated that they had never found evidences of infection prior to that of epithelial metaplasia. In a few cases they found metaplasia only, but usually this was associated with infection.

Capper (7) reported that chickens which lacked this factor were more prone than adequately fed controls to develop a disease known as visceral gout, and Kobashi (8) found that vitamin A deficient rats were more frequently and more seriously affected by a mite which burrows into the skin than others on complete rations.

Rats fed a diet containing a large amount of vitamin A (skim milk, wheat and butter) lived on the average twice as long as their litter mates on a diet low in vitamin A (skim milk, wheat and lard or cocoanut fat), according to the report of Sherman and McLeod (9). Lung disease was the commonest cause of death.

After a study of the diseases occurring in Malaya, Field (10) states that xerophthalmia and night-blindness—which both indicate vitamin A deficiency—are considerably more common among immigrant labouring Indians than among the Malays and Chinese. It is therefore likely that the diet of the Indians is deficient in this food factor. The resistance of the Indians to disease is also definitely lower than that of the other two races. For example; during the influenza epidemic in 1918 the mortality among the Indians showed a very marked increase whereas that of the Chinese and Malays was but slightly raised. The author therefore suggests that the lack of vitamin A is an important factor in the production of this low resistance to infection.

4. The determination of vitamin A rseerves in health and disease

This problem has been recently attacked in a concrete manner by several investigators (11) who have determined the amount of vitamin A present in the livers of individuals dying after accidents or from disease. It has been quite well established that the liver is the only organ in which vitamin A is stored to any appreciable extent, and therefore the determination of the amount present in it corresponds closely with the vitamin A reserve of the body as a whole. These authors extracted this vitamin from the liver and determined it quantitatively by means of the Carr-Price antimony trichloride technique. The first three observers measured the amount present in the livers of normal individuals that had died from accidents and they found very great variations, which could not be correlated with sex, age, etc.

Wolff and Moore were inclined to think that the variations were due to differences in the vitamin A content of the diets, and estimated that 16 per cent of the Dutch and 24 per cent of the English were subsisting on diets very deficient in this factor. This explanation, according to Fox, was not applicable to his results which were obtained in native African mine laborers whose diets had been standardized by the government. He suggests that the various individuals, although they appeared to be healthy were not able to absorb or to store this vitamin in a normal manner. He also suggested, however, that some of the very low readings might not be correct, and might be due to the presence of some inhibiter of the reaction, such as Morton had already described. The median values of the three series of accident cases were nevertheless very similar.

In the liver of very many individuals that had died from a variety of acute and chronic diseases a good supply of vitamin A was present, which would indicate that a store of this substance is no guarantee against infection. In several conditions the vitamin A titre was decidedly low according to one or more of these investigators—for example in organic heart or kidney disease, venous thrombosis, septicaemia, bronchitis and bronchiectasis. Affections which led to injury of the liver cells were frequently associated with low readings. In contrast to the other investigators Vogt concluded, after assaying the livers of 300 individuals, that sepsis does not deplete this organ.

Green found that the livers of gravid patients that died near term from non-septic causes contained but small supplies of this substance, and Wolff reported similar results in puerperal cases. The vitamin A concentration in the serum of such patients is also low (Wolff, and also Menken (12)). On theoretical grounds one would expect pregnant individuals near labour to be considerably depleted of this vitamin as a result of the demands of the growing foetus. However, the liver of a new-born infant usually contains only about one-quarter as much vitamin A as that of a normal adult, and also the concentration of this substance is very low in the sera of such infants.

Green also carried out similar assays on the livers of puerperal septicaemic patients, some of whom had been treated for long periods with large amounts of vitamin A concentrates. The average titre of the treated cases was considerably higher than that of the untreated, but both showed wide variations and usually it was found that only a very small percentage of the ingested vitamin had been retained in the liver. Apparently large amounts of vitamin A can be rapidly destroyed by the body, and the store in the liver is only gradually increased, even when excessive amounts are fed.

Menken's determinations of the vitamin A and carotene concentrations in the blood of sick and well individuals are also interesting. The highest titres were found in the well and their average was slightly higher than that of the sick. There also seemed to be some correlation between the titres and the economic status of the patients, as the readings were about twice as high among the better off as among the poor, which would indicate that the amount of this factor that is stored by the individual varies with the intake.

Clausen (13) measured the concentration of the caritinoids which includes carotene, the precursor of vitamin A, in the plasma of a large number of healthy children, and tried to correlate these readings with their susceptibility to colds. He inferred that only about 5 per cent showed a relative deficiency of vitamin A, but unfortunately some of the readings were quite anomalous. This author found that the caritinoids were lower during infections, expecially if these were severe, but this reduction was shown to be due largely to the lessened food intake. This suggested the possibility of feeding vitamin A in easily assimilable form during acute diseases. This was undertaken, and the results in these tests will be discussed later (page 154).

In an earlier report based on the study of the diets and respiratory infections of 700 children this same author showed that a relative deficiency of vitamin A may exist and be correlated with the severity of the infection. He also stated that the greatest number of severe infections in infants 6 to 24 months old were found in those that had not received cod-liver oil or vegetables, and whose plasma carotins were low. He also found that these readings were low in healthy children in the months of December, January and February, and that in infants that had received adequate amounts of cod-liver oil the plasma carotins were "nearly the same, whether or not mild or severe infections were present."

5. Susceptibility to artificially induced infections

(*a*) *Reduced resistance in vitamin A deficient animals.* Verder (14) fed vitamin A deficient rats (60 days on diet) and adequately fed controls suspensions of B. enteritidis, and after four to seven days killed them and made bacterial cultures from their organs. She found that many more of the avitaminic animals yielded positive spleen cultures and that in one small experiment two of the three deficiently fed rats died and the one control lived.

Ackert, McIlvaine and Crawford (15) infected chicks fed diets lacking in this factor and normal diets with the eggs of a chicken round worm, and after several weeks examined the birds for the presence of these parasites. They found a much larger number of longer worms in the malnourished chicks. The vitamin A deficient chicks were irradiated, whereas the controls were not, but received cod-liver oil.

In Boynton and Bradford's (16) work a bacillus of the mucosus capsulatus group was injected intraperitoneally into vitamin A deficient and control rats (deficient diet + cod-liver oil). When the animals were injected after they had been on the diets for four weeks, slightly more of the controls died, and after ten weeks all of both groups died. However, after six and eight weeks about half of the controls lived, and all of the inadequately fed rats died. The entire number of rats was forty-six.

In a brief communication Szulc and Kolodziejska (17) reported that following intraperitoneal infection with tubercle bacilli (human strain) 83 per cent of their vitamin A deficient rats showed symptoms indicative of inflammation of the respiratory system, which was confirmed by post-mortem examination. Smears which were made of the organs of some of the rats also showed B. tuberculosis. The controls that were fed the same diet plus radiostoleum remained in apparent good health, although they harboured the infection as three died of shock when injected with tuberculin at the conclusion of the experiment.

Finkelstein (18) found that the course of the infection, which followed the intraperitoneal injection of B. tuberculosis (bovine), was considerably more rapid in vitamin A deficient mice than in controls that were given carotene in addition to the diet. After 44 days all of the deficient, but only 30 per cent of the carotene fed mice were dead.

However, none of the latter group survived more than 74 days. The glands, lungs and spleens showed tuberculous involvement. Non-infected controls did not die during the experimental period.

According to the report of McClung and Winters (19), fifteen days after the intraperitoneal injection of mouse typhoid I, only about 5 per cent of their vitamin A deficient rats were alive, whereas about 95 per cent of the adequately fed rats survived with no symptoms until nine days after the infection when, for some unexplained reason, they were killed. The animals were infected as soon as the avitaminic group began to lose weight, which was after seven weeks on the diet, and only one of them showed a bloody discharge around the eyes at that time. Both groups were fed irradiated yeast, and the controls were given cod-liver oil as well.

Reiner and Paton (20) reported that a small group of rats (7 rats) on a diet devoid of vitamin A died more quickly after infection with a trypanosome than rats fed a complete ration.

No difference was found in the extent of the slight tuberculous lesions which followed the instillation of suspensions of bovine tubercle bacilli into the eyes and mouths of vitamin A deficient and normal rats (Harris and Griffith (6)). The former animals were given very small amounts of vitamin A at intervals in order to keep them alive. Apparently these hypovitaminic rats died after 20 to 93 days, and the controls survived and were killed later, but this difference is not considered to be of any significance by the authors. In a second test they found that such vitamin A poor rats showed more tubercle bacilli in their mesenteric glands after the repeated feeding of human tuberculous sputum than did the normal controls. As has been mentioned before, the rat is very resistant to tuberculosis infection.

M. R. Green (11) tested the resistance of a few rabbits on vitamin A free and normal diets to nasal instillations with Past. leptisepticum, and to intraperitoneal injections of type I pneumococci. When the former organism was used two of the four inadequately fed rabbits died with Past. leptisepticum septicaemia, whereas all of the controls lived. In the pneumococcus experiment all of the deficient animals died and half of the controls lived. The small number of animals used in these experiments detracts very materially from the significance of the results, which, however, are very promising.

In some recent experiments (21) the author has compared the resistance of rats without and with vitamin A (given as carotene) to an oral enteritidis infection. About one-third of each group was not infected and served as diet controls. When the animals were kept on the deficient diet for 5 weeks before infection some of the uninfected controls died eight days later, and the results were of no value. If the animals were infected after 3 weeks on the defective diet none of the uninfected rats died during the experiment, and the results were quite striking, as 90 per cent of the normal rats survived as compared with only 31 per cent of the deficient. In the first experiment (13 animals) all the deaths occurred within 29 days on the diet; and in the second (20 animals) all the deaths were within 41 days. The infections were therefore set up in the early stages of the avitaminosis. These results are being further confirmed.

Summary of experimental infections. In all of these experiments the use of a larger number of animals would have added much more weight to the conclusions. Also, in only two of the investigations were uninfected diet controls included. The positive spleen cultures which Verder obtained from her deficient animals after feeding B. enteritidis are probably, although not necessarily, associated with a decreased resistance. The larger number of longer round worms that Ackert found in his avitaminic chicks might be due entirely to slight alterations in the intestinal contents, and as a matter of fact this author stated that there was more material in the intestines of these chicks than in the normal birds. However it is more likely that the findings indicated a decreased resistance in the chicks.

The injection of a pharyngeal organism intraperitoneally (Boynton and Bradford) is without doubt an unnatural way of setting up an infection, and the deaths ensued very soon (within 48 hours) after the inoculation. In addition the results are inconsistent, as the decreased resistance of the inadequately fed rats, which was evident after eight weeks on the diet, was not demonstrated at ten weeks. It is very difficult to gauge such infecting doses correctly, and the older animals were probably given too much of the culture. The repetition of the experiment would perhaps have cleared up this discrepancy. As very few details of their experiments were given, Szulc and Kolodziejska's report cannot be properly evaluated. Finkelstein and also Reiner et al.

demonstrated that the deficiently fed animals died more quickly than the controls, but it is of little practical importance how quickly death supervenes if the outcome is always fatal.

McClung and Winter's animals were infected earlier in the course of the avitaminosis, when all except one of them appeared to be healthy. The choice of the infecting organism was not so fortunate, as it had to be given into the peritoneum and gave rise to a fulminating infection, but the difference in the resistance was however very marked. The author of this review has found that a closely allied organism, Salmonella muriotitis (enteritidis), is suitable for infecting such rats by mouth, if given in sufficiently large doses (about 3 cc.).

If Green could confirm her findings in larger numbers of animals her results would be very impressive.

The decreased resistance of the deficient animals in the experiments in which the infecting agent was fed or instilled into the nose could be explained on the assumption that the permeability of the nasal or intestinal mucous membrane was increased. This theory, however, would not explain the lower resistance of similar animals to infections introduced intraperitoneally. One would therefore conclude that the general as well as the local (epithelial) resistance of these deficient animals was depressed. Apparently also the resistance is reduced quite early in the course of the avitaminosis, before any signs of ill health are evident.

6. The use of vitamin A in infections

I. Experimental. After pyorrhoea has developed in dogs, due to the lack of vitamins A and D during the period of growth, the administration of vitamin A in large amounts may cause the arrest, but will not completely cure this disease, according to M. Mellanby (22).

In a recent report, Topley, Greenwood and Wilson (23) state that mice on a normal diet, which were given large additions of vitamin A, either as a concentrate or as carrots, did not survive in such large numbers after an experimental epidemic caused by B. aertrycke as did animals on the normal diet alone.

II. Clinical. (*a*) *Results indicating an increased resistance.* Two of the puerperal septicaemia cases which Mellanby and Green (24) reported in 1929 were treated successfully with a very concentrated preparation of vitamin A. In the preceding two years when other methods of treatment were used nine-tenths of their patients with similar infections had died.

Donaldson and Tasker (25) used this same vitamin A preparation in the treatment of 58 native labourers who were suffering from pneumonia. The results (8.6 per cent mortality) were not quite as good on the whole as those obtained by the use of both vitamins A and D (7 per cent mortality), but were considerably better than those produced by the routine treatment (13 per cent mortality). Donaldson also stated that he had found vitamin A of great value in the treatment of a number of cases of severe sepsis.

In 1932 Cioslowski and Szczygiel (26) reported that vitamin A concentrates (avoleum, B.D.H.) had very favourable effects when used as the sole treatment of five actuely ill post-partum and post-abortion infection cases. Blood cultures were positive in four of the five patients and all of them had fever of 104° F. or over. Two were complicated with pneumonia. The vitamin A was given by mouth, and the temperatures returned to normal in 13 to 19 days after the commencement of the treatment. These intervals were fairly long and it is possible that the natural resistance of the individual was the factor responsible for the recovery. That all of these patients did recover is, however, remarkable, as is also the courage of the investigators in treating such gravely ill individuals with this preparation alone.

Erben (27) treated 40 infants and children with a highly concentrated standardized vitamin A concentrate (Vogan) in 10-drop daily doses. Many of the infants were underweight, and the administration of this concentrate apparently led to rapid gains in weight. The incidence of catarrhal infections was not lessened, but their effects were less severe, as most of the infants did not lose weight and fewer of them showed really high fever. This concentrate, however, was of no value in cases of eczema or secondary anaemia, or in pyuria in older children.

(*b*) *Results indicating no increased resistance.* Wright and his coworkers (28) fed a series of infants a superabundance of vitamin A (as a concentrate) in an effort to reduce the incidence of respiratory infections. All of the children received one dessertspoonful of cod-liver oil daily and one group was given the concentrate in addition. The control children, therefore, received considerable vitamin A and the large additions of this substance that the other group received did not increase their resistance to respiratory infections and otitis media.

Barenberg and Lewis (29) compared the incidence of respiratory disease in infants fed skimmed or whole milk with and without viosterol or cod-liver oil. As considerable vitamin A is found in milk fat, these diets contained varying amounts of this factor. In addition butter (10 grams daily) and vegetables were added at 4 and 6 months respectively. Only six of the patients were fed on the skimmed milk diets and it is probable that all of the other diets contained ample amounts of vitamin A, and it is therefore not surprising that further additions of this substance did not alter the incidence of such infections.

Orenstein (30) has recently published the results of an extensive investigation on the effect of administering large doses of a concentrate of vitamin A to 375 native miners in South Africa who were suffering from pneumonia. A corresponding number of routinely treated pneumonia patients who were in hospital at the same time served as controls. The vitamin treatment had no effect on the mortality, hospitalization or complication rates.

In 1932 Prakken (31) reported that the treatment of "eczema seborrhoicum infantum" with vitamin A ("Davitamin-A") was a failure.

Clausen (13) administered carotene in oil (0.3 mgm. per kilogram body weight) to 63 cases of scarlet fever. Alternate cases did not receive this treatment and served as controls. There was no evidence of any beneficial effect either on the fever or on the number of complications, but, as this author states, a much larger series would be needed to settle this question definitely. He suspected that these patients were not suffering from any deficiency of vitamin A and that no good effect should therefore have been expected. However he did find that the plasma caritinoids rose about a week or ten days after the commencement of the carotene treatment, but the rise was not so marked if the cases showed complications at the onset or if the fever persisted for more than two weeks. He also determined the vitamin A titre of the plasma, and found that it also rose about two weeks after the carotene feeding was begun, and that it was also less in cases with continued fever. About five days after the drop in temperature it rose above the normal level, although it fell to a new low level about a week later. The significance of these later alterations is obscure, but there was some indication that the administration of carotene lessened this last fall. The point of particular interest is that complications or prolonged fever reduced the height of the rise of the caritinoids and of the vitamin A in the plasma. In an earlier report this same author found that in a series of rather severe otitis media cases a "rather rapid improvement" frequently appeared to follow the elevation of the carotene in the blood.

Hess and his confrères (32) have recently reported that added vitamin A (either as haliver oil or carotene) does not reduce the incidence of either winter or summer respiratory infections among infants. In addition, cases of pneumonia, bronchitis, pharyngitis, otitis media, conjunctivitis and skin infections were just as common among the babies fed these supplements as among those on the usual diets. All of the infants received viosterol as a source of vitamin D. They also collected evidence which showed that night blindness and xerophthalmia were rare on this continent. Their general conclusion is that our dietary is not deficient in vitamin A.

Summary of clinical infections. The very gravely ill post-partum or post-abortion septicaemia cases which Mellanby and Green and the Polish investigators treated so entirely successfully with vitamin A concentrates were very probably subsisting on diets partly deficient in this factor. Donaldson and Tasker's miners were, according to Butt (24), also living on diets low in vitamin A.

If Orenstein's much larger series of similar patients had been fed improved diets, this would, no doubt, have been mentioned. The latter's results when he treated pneumonia patients with vitamin A concentrates were entirely negative.

The children in Barenberg and Lewis', Wright's and Prakken's series were probably receiving enough vitamin A without any further addition.

It is very likely that diseases will be better resisted by patients suffering with all degrees of vitamin A deficiency if added amounts of this factor are fed, but if the diet already contains an abundance of this substance there is no reason to expect that an excess will increase the resistance. Unfortunately we do not know what is the optimum intake of this factor. The writer is of the opinion that vitamin A does not exert an anti-infective action per se, and that beneficial effects can follow its administration only when the patients have been subsisting on diets that are partially deficient in this substance.

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VITAMIN D

1. Variations in natural immune bodies and tissue reactions due to vitamin D

(*a*) *Results indicating that these are reduced in vitamin D deficiency.* Smith and Wason (1) reported in 1923, that the serum of rachitic rats had a considerably lower bactericidal effect on B. typhosus than that of normal rats, and that a similar although less marked difference was found when the bacteriotropic activities of the sera were compared. In the latter test the rat serum, and a mixture of human leucocytes, guinea pig complement and a staphylococcus suspension were used. When the rickets was cured by exposing the rats to ultraviolet light, the immune reactions were unchanged. Some of Smith and Wason's conclusions were later criticized by Findlay (2) on the grounds that even normal rats vary markedly in their reactions to B. typhosus. Findlay (2) himself reported that the serum of two-thirds of his rachitic rats showed a definitely lessened bactericidal action on staphylococci. He also found that if vitamin A and D deficient rats were exposed to ultraviolet light, the onset of the spontaneous infections and the coincident reduction in the bactericidal effect of the serum on staphyloccoci was delayed, which would suggest that vitamin D was a factor in maintaining the antibacterial resistance.

(*b*) *Results indicating that these are not reduced in vitamin D deficiency.* No difference was found in the complement titre of normal and rachitic rats by Smith and Wason (1). This finding was confirmed by Green in rabbits (3). She also showed that there was no difference in the natural agglutinins for B. typhosus or hemolysins for sheep's red blood cells in rachitic and normal rabbits.

(*c*) *Results indicating that these are increased with added vitamin D.* According to Holsen (4) daily doses of 1 per cent vigantol solution (irradiated ergosterol) increased the bactericidal effect of rabbit's blood on staphylococci. Hypervitaminosis D, on the other hand, decreased it. Using half grown rabbits as his test animals, Pfannenstiel (5) (1927) found that small doses of irradiated ergosterol raised the bactericidal action of their blood on typhoid bacilli. Larger doses caused severe intoxication and death.

2. Variations in acquired immune bodies due to vitamin D

(*b*) *Results indicating that these immune bodies are not altered.* Freund (6), in 1932, reported that large doses of vigantol (irradiated ergosterol) given both before and after, or only after the injection of sheep's red cells, did not increase the amount of hemolysin produced over that obtained after the same course of immunization in untreated rabbits.

Also, the production of acquired typhoid agglutinins and sheep's cell hemolysins and hemagglutinins is equally good in both rachitic and normal rabbits according to Green (4).

Summary of immunological investigations. The evidence of the various authors is extremely contradictory. Smith et al. found that the natural typhoid agglutinins were reduced in rachitic rats, whereas Green found them unchanged in rabbits suffering from the same deficiency. Findlay's suggestion that rats are unsuitable animals for such tests, as they vary greatly in their reaction to this antigen, may explain the discrepancy. Both Smith and Findlay found the bactericidal titres of rachitic rats' sera for staphylococci reduced, but the former reported that the exposure of the animals to ultra-violet light did not alter the readings, which would suggest that the lowering was not due to the rickets.

In normal animals small doses of irradiated ergosterol apparently increase the bactericidal titre both for typhoid bacilli and staphylococci. Large doses have the opposite effect and this may explain Freund's results (no increased hemolysins when large amounts of vigantol were fed).

Green's work, which is the most recent and painstaking and which was carried out on numerous rabbits, showed no reduced production of any of the antibodies in the rachitic animals. A similar investigtion, but including some of the antigens that Findley employed, might well be carried out on the rat.

3. Occurrence of spontaneous infections in vitamin D deficiency

(*a*) *Infections indicating a reduced resistance. I. Experimental.* In 1925, Manninger reported that after young pigs developed what he diagnosed as rickets, due to the feeding of a diet largely composed of barley, they very frequently developed rapidly fatal paratyphoid infections. If the diet was rendered even more unbalanced by the addition of large amounts of phosphorus, the disease developed earlier and death supervened more quickly. The same organism was commonly present in normal young pigs, but did not provoke any symptoms.

According to György, Jenke and Popoviciu (8), when an epidemic of coryza broke out among their experimental rats, 39 per cent of those that were suffering from rickets died, whereas all of those on the adequate diet survived.

Similarly, when an epidemic of paratyphoid appeared among Eichholz and Kreitmair's (9) rats, the rachitic animals died in large numbers (20 out of 24 died). The others which were fed the same rachitogenic diet plus 0.002 mgm. irradiated ergosterol daily, survived for the most part (2 out of 24 died). There was however some cannibalism among the rachitic animals before they were separated. These same authors had previously noted that growing hens or rats when fed rachitogenic diets were especially apt to develop infections.

II. Clinical. Respiratory infections have long been thought to be abnormally common in rachitic children (10). After analysing a series of 386 postmortem examinations, Schmorl (11) stated that 90 per cent of the children that died under four years of age showed rickets, which probably indicates that such individuals have a low resistance to infection. However, as no statement was made as to incidence of rickets in the whole population of children in this age group, this deduction can not be proved.

Bromer (12) studied a series of rachitic children in 1930, and concluded that in severe cases, in which the chest wall lost its usual rigidity, respiratory infections were very frequently fatal. On the other hand, when mild rachitic deformities of the chest were present, the death rate following pulmonary infections was not higher than usual.

According to Warner (13), a lack of vitamin D is associated with rheumatic fever in older children. This disease is much commoner in the winter and spring months than in the rest of the year, and this author also found that in these cases there was a deficiency of calcium in the blood and spinal fluid.

As psoriasis apparently does not occur in the tropics, Monash (14) suggests that a deficiency of vitamin D may be an etiological factor.

The fact that he found that 15 to 20 drops of viosterol daily improved these cases adds some support to his theory. Local applications were however also used.

Many individuals have volunteered the information that the taking of vitamin D renders them less susceptible to colds (15), but whether this effect would follow such a régime in all individuals has not yet been proved.

(*b*) *No increase in infections.* Mellanby (16) found that some bronchopneumonia occurred among dogs fed oxidized cod liver oil or butter which contained vitamin D but no vitamin A. Animals given untreated cod liver oil or butter escaped this infection. He concludes that vitamin A and not vitamin D conferred the protection. Dogs which lacked vitamin D did however show collapse of the lungs due apparently to the inadequate functioning of the bones and muscles.

Barenberg, Greene and Abramson (17) compared the incidence of pneumonia in a series of rachitic and non-rachitic children, 6 to 18 months of age, all of whom were living in an institution. The rachitic children showed slight but definite beading, and cupping and fraying of the epiphyses. Most of the non-rachitic children were protected with cod liver oil, although a few were treated by an ultra-violet lamp. Seventeen of the 102 non-rachitic children developed pneumonia, whereas only four such cases appeared among a similar number of rachitic individuals.

Summary of spontaneous infections. Practically all of the evidence, with the exception of Mellanby's dogs, which may not have been suffering from true rickets, points to an enhanced susceptibility to infection in animals with marked rickets (pigs, rats and infants). Whether this same phenomenon is associated with mild degrees of rickets, such as were present in Barenberg's patients, is not proved as yet. The supply of vitamin D in northern climates in the winter time is very low and this may be associated with the seasonal incidence of respiratory infections.

4. *Susceptibility to artificially induced infections*

(*a*) *Reduced resistance in vitamin D deficient animals. I. Rachitic animals.* When Grant and her co-workers (18a) kept their breeding mother rats on diets devoid of vitamin D, they found that the fourth and fifth litters regularly, and even the first litter in cold gloomy weather, showed rickets. Their normal rats were very resistant to infection with a virulent strain of B. tuberculosis (1 out of 23 showed tuberculous lesions in the lungs). In contrast to this, about a third of their rachitic rats developed such lung lesions after a similar infection. It is possible that the condition with which Grant was dealing was not rickets, as most authors are agreed that the absence of vitamin D alone in a rat's diet will not result in this disease.

In her later work (18b) this same author found that when cod liver oil was fed ad lib. to the offspring of mothers that were on a rachitogenic diet, although rickets did not appear, tuberculous infections could still be induced. In fact, this investigator goes so far as to say that the addition of the cod liver oil rendered the rats more susceptible to tuberculosis, but this statement is based on very slight differences. The young rats were injected within one week after weaning and had therefore had the benefit of the cod liver oil for this short period only.

Eichholz and Kreitmair (9) described two experiments in which the resistance of adult mice on normal, rachitogenic, and rachitogenic plus vitamin D diets, to a highly pathogenic strain of pneumococci given intraperitoneally was compared. In the first test about half of both the normal and those given the vitamin D additions survived and all of the rachitic mice died. In the second test only four of the fourteen rachitic mice survived as compared with about half of the nonrachitic.

The minimum lethal dose of suipestifer bacilli (intraperitoneal) for young rats on normal and rachitogenic diets with and without the addition of cod liver oil or irradiated ergosterol was determined by Maurer and Hofmann (19). The normal rats survived about eight times the dose that killed the rachitic ones and when cod liver oil or irradiated ergosterol was added to the rachitogenic diet, the lethal dose had to be multiplied four to six times. Direct irradiation did not raise the resistance of the rachitic rats to any extent. Comparatively few animals were used in all these tests but the differences found were usually very marked.

Ackert and Spindler (20) reported that the lack of vitamin D decreased the resistance of chickens to an intestinal nematode. The results in only one of their four experiments were clear cut, although the others showed slight differences in the same direction. The vitamin D was supplied in the form of 2 per cent aerated cod liver oil. In the best experiment, 30 per cent of the vitamin D deficient chicks had freed themselves of the parasite by three weeks after parasitization, whereas 80 per cent of the chicks fed vitamin D had been able to do so. When birds on the rickets-producing diet were irradiated with a mercury vapour lamp, the elimination of the worms was no better than in the rachitic chicks.

M. R. Green (3) tested the resistance of rachitic and normal rabbits to nasal instillations with a virulent strain of Pasteurella lepisepticum, which is probably the etiological agent of acute rabbit snuffles. Eight of the twelve rachitic rabbits died. Although they showed consolidation of the lungs, the instilled organism was not recovered from three of these dead rabbits. Nevertheless, it is probable that the deaths were also due to the pasteurella infection. Only three of the eleven normal controls died. Intranasal instillations with a relatively avirulent strain of pneumococci were also tried and the course of the disease was followed by means of daily blood cultures taken from the ear vein. The morbidity, as measured by the presence of pneumococci in the blood stream, was very much higher (83 per cent) in the rachitic than in the normal rabbits (33 per cent). The mortality was also higher, as 42 per cent of the 12 rachitic and only 16 per cent of the 12 normal rabbits died.

The author of this review, in collaboration with Ross (21), published in 1930 a report of experiments in which the resistance of rachitic and non-rachitic rats to oral infection with a highly virulent strain of Salmonella enteritidis was compared. The vitamin D was provided for the non-rachitic rats by the irradiation of one-third of the otherwise identical diet. The rats were observed for 28 days after the infection and it was found that only 5 per cent of the 89 rachitic rats survived in contrast to 30 per cent of the 94 rats receiving vitamin D. In the majority of the dead rats the specific organism was recovered from the heart's blood. Uninfected diet controls should have been included in this series, although deaths from rickets do not occur usually until after nine weeks or more on this diet. These same authors confirmed these general findings in 1932. Steenbock's rachitogenic diet fortified by additional amounts of vitamins A, B, C and E and containing 12.5 per cent of dried white bread was fed to the rachitic animals, and this maintained their nutrition fairly well. The non-rachitic rats were given 12.5 per cent of vitamin D containing bread instead of the ordinary white bread. The technique of infection remained as before and it was found that 22 per cent of 147 rats fed the rachitogenic diet survived as compared with 57 per cent of 151 rats fed the same diet with the addition of vitamin D. These results were statistically significant.

II. Non-rachitic animals. The mortality (63 per cent) among adult rats that were fed a diet deficient only in vitamin D after an intraperitoneal mouse typhoid infection was considerably higher than that among the same number of rats fed a similar but adequate diet (27 per cent) according to the work of McClung and Winters (22). Forty-four rats in all were infected, and all the control rats looked healthy when they were rather prematurely killed on the ninth day.

The author of this review (23) has recently kept rats on complete diets and diets deficient only in vitamin D for three months and then infected them per os with several cubic centimeters of a broth culture of S. enteritidis. Forty-one per cent of those on the complete and 13 per cent of those on the deficient diet survived. These results were based on only 76 animals. If the animals are infected after only one month on the diets, there is very little difference in the survival rate, although in every one of five experiments made up of 188 animals, slightly more (2 to 12 per cent) of the animals fed the complete diets lived. About seven Steenbock vitamin D units per 100 grams of food were present in the complete diet.

(*b*) *No evidence of increased resistance.* According to Boynton and Bradford (24) rickets does not reduce the resistance to infection. They fed 22 rats on Steenbock's rickets-producing diet and a similar number on the same diet with 0.0015 mgm. of irradiated ergosterol daily in addition. Groups of the rats after 4, 6 and 8 weeks on the diets were injected intraperitoneally with a bacillus of the mucosus capsulatus group. Death supervened rapidly, usually in less than 30 hours. In the tests carried out at 4 and 6 weeks, equal numbers of both groups died, but at 8 weeks all the rachitic rats died and only one-half of the non-rachitics. The diagnosis of rickets was made on the gross anatomical changes only, and the authors state that rickets was evident in the "majority of the animals."

Lassen (25) placed four rats on McCollum's rachitogenic diet and four on a normal diet. Four weeks later the animals were all fed a moderately virulent strain of paratyphoid bacilli (Breslau), the normal rats receiving twice as large a dose as the rachitics. One rat from each group was killed on the second, fifth, twelfth and nineteenth days and many of the organs were cultured. Three of the rachitic animals showed somewhat more marked infections than the normal controls, although the author considered this difference negligible. Although this method of investigation is valuable in the study of the mechanism of infection, it does not provide any measure of the degree of natural resistance.

As the mice which lacked vitamin D in Finkelstein's (26) experiment received twice as much carotene (provitamin A) as the ones that were given vitamin D, it is difficult to decide what effect the latter vitamin had on the survival time after intraperitoneal infection with B. tuberculosis. The author concluded that vitamin D was of little importance in determining the course of such infections in mice.

Summary. In experiments such as Grant's, it is difficult to be sure that all the tuberculous lung involvement has been discovered, but as about seven times as many of the rachitic rats showed these lesions as of the controls, her conclusions are probably justified. The infections which both Eichholz and Maurer used were very unnaturally produced and very rapidly fatal, but the difference found in the survival rate was very definitely in favor of the non-rachitic animals. Ackert and Spindler's results might possibly be explained by alterations in the intestinal contents, although this seems unlikely, and it is more probable that the rachitic rats were unable to cope with the worm infestation as well as the adequately fed controls. The author of this review is more favorably disposed towards the natural type of infection such as Green and Robertson and Ross used. Due precautions were taken by these authors to exclude the possibility that the bacteria that were to be used were already present in the animals before the artificial infections were set up. Green's experiments included too few animals, but much larger numbers were used by Robertson and Ross. All of these investigators found a considerably lower resistance in the rachitic animals.

More experiments should be undertaken to prove conclusively whether the absence of vitamin D in an otherwise adequate diet is associated with an increased susceptibility to infection, as McClung and Winter's and the author's recent experiments seem to indicate.

One would accept Boynton and Bradford's negative results with more assurance if a more detailed examination for rickets had been undertaken. It is usually conceded that at some times it is difficult to get uniformly severe rickets in rats, and this may have been the case in their experiment. After eight weeks on the diet the rachitic rats were definitely more susceptible.

The great majority of these investigators have, however, shown that animals lacking vitamin D are more susceptible to artificially induced infections.

5. *The use of vitamin D in infections*

(*a*) *Results indicating an increased resistance. I. Experimental.* In 1930, Pfannenstiel and Scharlau (27) observed that the infection of a rabbit's skin with hemolytic staphylococci caused a marked inflammatory reaction, which took about one month to heal. If small doses of irradiated ergosterol (vitamin D) were fed, the abscess was smaller and more sharply defined, and healed in about two weeks. Similarly, Freund (6) fed large rabbits varying amounts of vitamin D (vigantol, 5 to 20 drops daily) for varying periods of time (5 to 31 days), and then set up a local inflammation, either by dropping mustard oil into the conjunctival sac or by injecting terpichin (a turpentine derivative in olive oil with a little quinine and anaesthesin added) or hemolytic streptococci into the skin. The inflammatory reactions were considerably less intense and usually subsided more quickly in the animals receiving the extra vitamin D. In rats and mice however, large doses of vigantol, about 20 drops daily for 9 to 15 days, did not lessen the reaction which followed the subcutaneous injection of streptococci.

Two groups of adult guinea pigs, infected subcutaneously with 0.1 mgm. of virulent human tubercle bacilli, were fed large (20 drops) and small (5 drops) daily doses of vitamin D, as vigantol, for 20 days by Theiss (28). A third group infected in the same way received no treatment and served as controls. When the animals were killed 65 days after the infection, it was found that those that were fed the small amounts of irradiated ergosterol showed very much less tubercle infection than the others. Occasional calcified tubercles were found in a few of the rats fed the vitamin D, but this was apparently not the process that checked the spread of the tuberculosis in the group fed the smaller doses.

In Spies' (29) series, twenty-five rabbits were infected intravenously with a highly virulent strain of bovine tuberculosis, and fourteen of them were given several doses of 3 to 5 cc. of a concentrated solution of irradiated ergosterol which had a vitamin D potency 1000 times that of cod liver oil. About half of the remainder were given corresponding amounts of the inert oil solvent and the rest received no treatment. All of the rabbits were dead within twenty-four days, but only those that were fed the vitamin D showed calcification in the necrotic and caseous tubercles in the lungs. About half of these also showed calcification in the tubercles in the kidney. However, in five of the rabbits calcification in the aorta, and in two, calcification in some of the renal tubules and vessels was demonstrated also, which would suggest that such treatment should be used with caution in human tuberculosis.

Policard and his co-workers (30) reported that 5 mgm. or more of irradiated ergosterol daily led to the calcification of tuberculous foci in large guinea pigs.

Four guinea pigs were infected intravenously with a human strain of B. tuberculosis by Meerssenan and Tricault (31). The two untreated controls died in 14 and 27 days, whereas a third that was given 3 drops (0.5 mgm.) of an oily solution of irradiated ergosterol daily from the fifteenth day after infection lived for 40 days, and the fourth which received this medication continuously after the infection lived for 105 days.

Levaditi and Po (32) showed that when non-toxic doses of irradiated ergosterol (2 to 10 mgm. daily per kilogram body weight) were fed to rabbits, marked calcification occurred in the orchitic, pulmonary and liver tubercles which had been induced by the intratesticular inoculation of human tubercle bacilli. That the dose was non-toxic was evident from the fact that there was no calcification in the aorta or kidney tissues. These same authors later used a bovine strain and obtained similar results, as the three rabbits fed ergosterol all showed calcification, whereas of three untreated controls, only one showed this reaction.

In a third series, they injected 5 mgm. of B.C.G. into the testicles of eight rabbits. Two weeks later half of them were started on 2 mgm. of irradiated ergosterol per kilogram body weight daily. At the end of forty-two days, they were all killed and after examination their organs were assayed for calcium. All of the animals had tuberculous lesions in the testicles, but none of the untreated animals showed calcium in these organs. In contrast to this, two of the vitamin D treated rabbits showed considerable calcium (++), and the other two showed a trace of calcium (+) in the testicles.

Calcification of the lungs in both tuberculous and normal animals is said by Simonnet and Tanret (33) to follow the administration of irradiated ergosterol.

Rossi (34) described a uniformly fatal, diarrhoeal disease that attacked calves in the first few days of life. Before trying out the vitamin D treatment, 140 calves in succession had died from the disease. In the first case treated, 10 drops of irradiated ergosterol were given on 2 days. Diarrhoea appeared on the fourth day and caused death at the end of the first week. In the rest of the animals, 20 to 30 drops daily were given from birth for at least 5 days, and although numerous calves showed some diarrhoea, they all recovered quickly and completely. The ergosterol was given as an oily solution, and according to the author the dose used contained only a fraction of a milligram of irradiated ergosterol. It was however 6 to 10 times greater than the usual therapeutic dose calculated according to the relative weights of rats and calves. A similar disease among new born pigs was also successfully treated with the same solution in smaller doses.

II. Clinical. The effect of the administration of irradiated ergosterol in clinical tuberculosis has also been tested out by several observers. For example, Meerssenan and Tricault (31) reported that the administration of 30 drops (0.5 mgm.) daily of 0.1 per cent oily solution of irradiated ergosterol led to rapid improvement in ten out of eleven cases of pleural, peritoneal and glandular tuberculosis.

When Menschel (35) gave cases of open pulmonary tuberculosis 5 drops of a 1 per cent solution of irradiated ergosterol once or twice a day, he found that the expectoration and the hemorrhages were reduced and that the patients gained more weight and showed lower temperatures.

This treatment was however of no value in intestinal tuberculosis. It was of benefit in tuberculosis of the lungs, bones, pharynx and skin.

Massive doses of irradiated ergosterol (20 drops of a solution with a cod liver oil coefficient of 10,000) if given for from 5 to 10 days will usually produce hypercalcemia, according to Crimm (36). The hypercalcemia frequently persists for several months and can then be produced again by the readministration of the drug for a shorter interval. In one patient, after 270 days of hypercalcemia, roentgenological evidence was obtained of beginning calcification in new lesions. In another patient symptoms of an intestinal infection, possibly due to tuberculous lesions, which appeared during a period in which the blood calcium had dropped back to normal, disappeared after ten days of further treatment with vitamin D. The hypercalcemia which Crimm induced was very striking, but whether this favorably influenced the healing was not adequately established.

Pattison (37) fed $\frac{1}{2}$ pint daily of irradiated milk to patients with active bone tuberculosis and reported that marked improvement not only in the local lesions, but also in the general health occurred. Practically all of the cases had already been treated for several months in the hospital without much improvement.

Birkholz (38) found that vigantol (irradiated ergosterol) in high doses had a good effect in cases of ozaena, and Jakobsen (39) reported that 2 to 4 mgm. daily of the same preparation of vitamin D led to a rapid cure in two severe cases of infantile eczema. In addition, a 5 per cent pyrogallol triacetate salve was applied locally. In an older child, 8 mgm. daily of vigantol was used with equally beneficial results. Franke (40), in 1929, stated that he was able to cure a case of bronchial asthma with vigantol.

In the same year Starck (41) reported that when cases of ozaena were fed 4 to 8 mgm. of vigantol daily, the foetor and in most cases the crust formation also was rapidly cured.

After viosterol (250D) had been given in 10- to 20-drop doses daily for a month to 35 patients affected with acne vulgaris, Doktorsky and Platt (42) found that 75 per cent (actual count) of the pustules had disappeared in nine-tenths of the subjects. In the remainder, about half of the pustules were cleared up.

It has been demonstrated by M. Mellanby and Agnew, Agnew and

Tisdall that vitamin D plays an important part in the prevention of dental caries. However, as the relation of infection to this disease is at present uncertain, this aspect of the subject will not be discussed in this review.

(*b*) *Results indicating no increased resistance. I. Experimental.* The administration of vitamin D alone (irradiated ergosterol) did not lessen the extent of the lung lesions which followed the intravenous injection of tubercle bacilli into rabbits in the experiments of Pfannenstiel and Scharlau (43). However, the simultaneous administration of vitamins B and D was very beneficial.

II. Clinical. In Smellie's (44) work, one-half of the children in an open-air school were fed $1\frac{1}{4}$ pints of irradiated milk daily and the rest received the same amount of untreated milk. The children were tuberculous contacts, or were suffering from anemia, asthma, malnutrition, etc. When the progress of the two groups was compared, it was found that the children fed the non-irradiated milk had gained a little more in weight, but that in every other way the two groups were very similar.

Kramer, Grayzel and Shear (45) divided 18 tuberculous children (17 bone and 1 skin tuberculosis) into two equal groups. One group received 4 mgm. of irradiated ergosterol for four months, followed by 7 mgm. for the next eight months. The other group received no special medication, and both groups were fed a well balanced diet. It was found that the increased intake of vitamin D did not produce "any detectable acceleration of the healing process," nor did it give rise to any deleterious effects.

According to Gehrt's (46) results, vitamin D was of no value in the treatment of eczema.

Although Armand, Delille and Bertrand (47) admit that irradiated ergosterol is very efficacious in the treatment of the bony abnormalities of active rickets, they state that it does not affect the tendency to infection.

The administration of vitamin D alone or combined with vitamin B had no beneficial effect on sanitorium cases of tuberculosis according to the work of Scheurlen and Orlowitsch-Wolk (48).

Schaferstein (49) found that the course of tuberculosis was unchanged by vitamin D therapy.

Summary. According to both Freund and Pfannenstiel et al., the administration of vitamin D decreased the reaction and hastened the healing of skin lesions due either to bacteria or to chemical irritants. The fact that both the size and the duration of the lesion were reduced adds weight to the result.

In some of the reports reviewed, the action of this vitamin in experimental tuberculosis can be explained by its increasing the calcification. In others, the beneficial result appears to be independent of this process. For instance, Theiss found that small doses of irradiated ergosterol seemed to limit the spread of tuberculosis, whereas large doses did not have this effect, and some of the guinea pigs fed both doses showed calcified tubercles. Also, Meerssenan et al. found that vitamin D quite materially prolonged the lives of tuberculous guinea pigs, although no mention was made of calcification of the lesions. However, as four animals only were used in the experiment, and it is well known that tuberculosis runs a variable course in the guinea pig, these results should be interpreted with caution. Both of these experiments suggest that vitamin D has a beneficial effect on tuberculosis apart from its effect on calcification, but the proof submitted is not convincing.

Levaditi and Po found in three different experiments that doses of irradiated ergosterol that did not cause abnormal calcification in the aorta or kidney tissues resulted in calcified tubercles. Such lesions were not found in the untreated controls except in one isolated case. A rapidly fatal tuberculous infection was set up by Spies in order to avoid the natural calcification of the lesions. His doses of vitamin D were apparently on the border-line of toxicity, as about one-third of his treated animals showed calcification in the aorta and a few also had similar lesions in the renal tubules and vessels. Calcification of the tubercles was only seen in the animals that were fed this vitamin. It is well known that large doses of vitamin D cause pathological calcification in many organs, and that this occurs more readily in some species of animals than others. Levaditi and Po apparently used doses that stimulated calcification in the tubercles, but that were so small that they did not cause pathological calcification elsewhere.

The most obvious explanation for Pfannenstiel's results, in which he found that vitamin D was only of benefit in experimental tuberculosis when vitamin B was added also, would be that the vitamin B was too low in the original diet. However, other observers (9) also have noted that these vitamins in combination may exert a very favorable effect.

The clinical tests have given varying results. For instance, Meerssenan, and also Menschel, using small doses, report favorable results in pulmonary tuberculosis, whereas Scheurlen could not confirm this. Bone tuberculosis was apparently benefited by the small amounts of this vitamin that Menschel administered, but the large doses used by Kramer and his co-workers were without effect. Crimm's results suggested that large doses were helpful in tuberculosis of the lungs, but as no pathological examinations were made, it cannot be concluded that calcium was not deposited in non-tuberculous tissues also. One can probably not assume, however, that small amounts are valuable in the treatment of tuberculosis and large ones harmful. It is very often difficult to compare the various doses used, as they are expressed in several different units. Pattison used irradiated milk with advantage in cases of active bone tuberculosis, but Smellie found this same substance to be without effect in preturberculous and malnourished children.

The fact that such conflicting results have been obtained should stimulate further enquiry into these questions.

6. Ultraviolet light and infections

It has been well established that a comparatively narrow band in the ultra-violet region of the spectrum has the property of converting ergosterol into vitamin D. When man or the higher animals are exposed to such rays, the ergosterol which is present in their superficial tissues apparently undergoes this conversion and more of this vitamin thus becomes available to the organism. An extensive discussion of the large literature that has accumulated on the effect of light on the immunity reactions and on natural and experimental infections is beyond the scope of this paper, but some of these reports may be reviewed in passing, as in many instances the results have been ascribed to vitamin D. As far as the author is aware, no experiments have been carried out in which the effect of the antirachitic rays alone on infections has been determined, and the apparatus necessary for such an investigation would be large and costly. One is therefore confronted with the virtual impossibility of isolating the effect of these antirachitic ultra-violet radiations from that of the other rays and the other environmental factors (e.g., fresh air) associated with them, except in experiments in which the effect of light with and without these rays is compared. Using the latter method, Balderry (50) found that their presence or absence in sunlight did not alter the progress of gland or bone tuberculosis. That light devoid of the antirachitic rays is of therapeutic value has been shown by several investigators (51). For example, Ross, Tisdall and the author of this review (52) showed that sunshine that had passed through common window-glass and was therefore no longer capable of curing rickets nevertheless enhanced the resistance of rats to a paratyphoid infection.

Some sources of light however do emit much of their energy in the "antirachitic region," for example, the air-cooled quartz mercury vapour lamp, and some of the experiments in which such radiations have been used will be outlined below. Whether the immune reactions are influenced by such exposures is an open question. Hansen, Potthoff, Eidinow, Bessemans, Huntemüller and their co-workers (53) found that such treatment increased the antibody response, whereas Albela, Hartley, and Hardy and Chapman (54) reported that no such increase occurred.

The results that were obtained when the relation of irradiation to experimental infections was studied are also very conflicting. Several authors have found that the exposure of animals to light that is largely ultraviolet increases their resistance to infection, for example Mayer (55), working with tuberculous guinea pigs, Hill and Clark (56) with rats injected with pneumococci, when the irradiation preceded the infection, and Hansen (57) with typhoid vaccine in rabbits. In contrast to these, L. Hill et al. (58) with pasteurellosis in mice, Steinbach and his associates (59) with tuberculous guinea pigs, Hardy and Chapman (60) with various infections in rabbits, and others have reported negative results. Most of the irradiation carried out in the hope of reducing the incidence of common colds has proved to be of no value (61), although Maughan (62), L. Hill and Laurie (63) and Cassie and Cox (64) using small dosages have obtained favorable effects.

At the present time it is almost impossible to assess the results reported, but it would appear that small doses exert a more favorable influence than large ones. Much more investigation will be necessary before any reasonable judgment on the effect of the antirachitic ultraviolet light in infections can be formulated.

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VITAMIN B

1. *Variations in the natural immune bodies or tissue reactions in vitamin B complex deficiency*

(*a*) *Results indicating that these are reduced.* In 1922, Biondo (1) found that there was a moderate and progressive diminution in the opsonic index for anthrax bacilli in vitamin B deficient pigeons. About the same time, Findlay and MacKenzie (2) stated that the phagocytic indices, using both staphylococci and B. coli, were slightly lower in B. deficient than in normal rats. After three weeks on the diets, the difference was small, and after a similar additional period, it was further reduced but was still in favor of the normal animals. In the next year, reports of three investigations were published which indicated that at least some of the natural immune reactions were weaker in such deficiently fed animals than in controls fed normal diets. In brief, the results were as follows: Werkman (3) found that the phagocytic index of a vitamin B deficient rat for B. typhosus when determined in vivo was slightly reduced; Smith and Wason (4) reported that the bactericidal action on the same organism, and the bacteriotropic (opsonic) activity for staphylococci in the presence of heterologous leucocytes and complement was also lower than normal, and Findlay (5) demonstrated that the peritoneal exudate of vitmin B deficient pigeons had less bactericidal effect on pneumococci than that of similar birds on an adequate diet. Two years later, the last author, in collaboration with MacLean (6), showed that the blood of rats lacking vitamin B had a lessened bactericidal effect on staphylococcus aureus (slide cell method). According to Corbia (7) the opsonic index for typhoid and dysentery bacilli and for staphylococci is reduced in beri-beri.

(*b*) *Results indicating that these are not reduced.* However, some of these same authors showed that other immune reactions were unaffected by this dietary deficiency. For instance, Werkman (3) reported that when the opsonic or phagocytic indices were determined in vitro, no difference could be discovered between the deficiently and adequately fed rats, and Smith and Wason (4) found no change in the complement titre of such rats.

2. Variations in acquired immune bodies due to vitamin B complex deficiency

(*a*) *Results indicating that these immune bodies are reduced.* Although it is difficult to be sure exactly what diet Guerrini (8) used, he apparently compared the agglutinin production after several doses of B. coli vaccine of pigeons fed whole or husked rice. Although the diet of the birds fed the polished rice was chiefly deficient in vitamin B, other inadequacies were also certainly present. His results were however most striking, as the titre of the serum of the birds on the whole rice, set up against the homologous strain, was about $\frac{1}{9000}$, whereas that of the deficient birds was never over $\frac{1}{150}$. Setti (9) also found that birds fed normal diets developed higher immunity after vaccines than those that lacked vitamin B.

Vitamin B deficient rats immunized with B. typhosus also showed, when the phagocytic indices were determined in vivo, lower figures than normally fed rats that had been similarly immunized (Werkman (3)).

In 1928, Blackberg (10) reported that rats lacking vitamin B did not produce either agglutinins or bacteriolysins for B. typhosus as well as rats on a normal diet. When they were immunized with several injections of killed organisms the avitaminic animals showed much lower titres than the controls, but if living cultures were used, especially if in large doses, the difference was less marked. Blackberg suggests that the bacterial cultures contained small amounts of the missing vitamin.

Abderhalden and Wertheimer (11) stated in 1922 that after sensitization with serum more vitamin B deficient than normal pigeons died from anaphylactic shock after the second injection, and Wedgewood and Grant (12) found that if a young rat was deprived of this vitamin, it could be sensitized by the injection of a foreign protein and later killed by anaphylactic shock, whereas similar rats on a normal diet could not be so sensitized.

(*b*) *Results indicating that these immune bodies are not reduced.* Rats fed diets lacking in vitamin B, after the injection of typhoid bacilli, produce agglutinins and amboceptors as well as those on normal diets according to the work of Zilva (13) which was published in 1919.

Werkman (14) later (1923) reported that rats in the advanced stages of this avitaminosis produced agglutinins, precipitins, hemolysins and bacteriolysins as efficiently as the controls on complete diets. The same author (15) in the next year found that this avitaminosis did not interfere with the animal's capacity to form antitoxins.

Summary of immunological investigations. The process of phagocytosis, including the opsonins associated with it, seems according to several investigators (Biondo, Findlay, Smith and Wason and Werkman) to be depressed in non-immune vitamin B deficient animals. The last author found a lower phagocytic index only when the determinations were made in vivo and not when they were carried out in vitro. Reduced bactericidal effects were also elicited in these inadequately fed animals (Smith and Wason and also Findlay). The significance of the complement titre is probably unknown, but the former authors found it unaltered in vitamin B avitaminosis.

When immune animals were used, Blackberg found that the titres of agglutinins and bacteriolysins which followed the injection of dead cultures of typhoid bacilli were much lower in the deficient rats. This finding was quite the reverse of those already reported by Zilva and by Werkman. Guerrini's pigeons, whose agglutinin production was so low, could not accurately be called vitamin B deficient, although they were especially lacking in this factor. The increased ease with which anaphylactic shock could be induced in animals lacking vitamin B suggests that a marked alteration in the tissues of the body is present in this deficiency.

3. Occurrence of spontaneous infections in vitamin B complex deficiency

(*a*) *Infections indicating a reduced resistance.* Although it is sometimes stated that a deficiency of the vitamin B complex does not render the animals susceptible to spontaneous infections, quite a number of investigators have provided evidence to the contrary. For example, Findlay (16) noted that the tissues of animals fed vitamin B deficient diets were frequently attacked by bacteria, and suggested that such animals had partially lost their ability of ridding themselves of harmful organisms. The work of McCarrison is very impressive, but unfortunately most of his deficient birds were fed on polished rice, which as has been noted above lacks several other factors besides vitamin B.

However, he found that pigeons on this polished rice diet were very prone to become infected with B. suipestifer (17), and even although they were taken off this very poor diet and kept on a normal diet for some weeks, many of them showed the lesions of epithelioma contagiosium (18). The controls on normal diets which were intimately exposed to these sick birds very rarely became infected.

The symmetrical distribution and the absence of acute inflammatory signs would suggest that the skin lesions which Cowgill (19) observed in his vitamin B deficient dogs were probably not of infectious origin. A remarkable demonstration of the marked susceptibility to infection of pigeons suffering from this avitaminosis was provided by Barlow (20) who found that such birds very often showed a bacteremia, probably of intestinal origin, which rapidly cleared up when vitamin B (yeast extract) was administered.

Clausen (21) suggests that a deficiency of vitamin B may increase the susceptibility to intestinal infections, and Sherman and Smith (22) postulate that it may cause an impairment in the protective resources, resulting in mucous membrane infection at first and in systemic infection later.

Cody (23) found that rats that were kept on a vitamin B deficient diet showed a polycystic appearance of the nasal mucosa in the upper posterior ethmoid region. He also stated that there was a somewhat similar clinical syndrome due to the lack of this vitamin. This was characterized by slight but frequent postnasal discharge, and could be improved but not cured by the exhibition of brewer's yeast.

The statistical study of Maurer and Tsai (24) pointed to a correlation between the constant high human infant mortality under one week of age and the lack of vitamin B in the maternal diet during pregnancy and lactation. In their experimental work they found a very high death rate among young rats whose mothers were fed inadequate amounts of vitamin B during gestation or lactation, and they also noted that labor was difficult in such mother rats. Moore and Brodie (25) reported substantially the same results in their animal work, and also described a fatal human case in which a very young infant died with jaundice, fever, hematuria, diarrhoea, etc. The mother's diet had been very poor and one could not lay all the bad effects at the door of vitamin B. Also the presence or absence of an infection in the child was not proven, although it appeared to be likely.

Bray (26) found that the young breast-fed infants on the Pacific island of Nauru very frequently died of acute beri-beri. The mothers' diets were very deficient, often consisting merely of sugar and water. The infants that did survive for the first twelve months of life, but whose diets were low in vitamin B, suffered from very numerous infections. Public health measures were adopted which provided yeast both for the mothers and the babies, and since that time there have been very few infections, all of them mild, among the infants.

4. Occurrence of spontaneous infections in vitamin B1 deficiency

Whether the very high infant mortality (70 to 100 per cent) which Sure (27) found followed the transference of mother rats after parturition from normal to vitamin B1 deficient diets was due wholly or even partially to infections is problematical. The dead young rats showed hemorrhages in the intestine and osteogenetic system, and death was often preceded by spasms and paralyses. Sure suggests that many of the deaths among human infants under one year of age associated with gastro-intestinal disease may be due to this vitamin deficiency. It is well established that the lactating mother rat needs four to five times the amount of vitamin B that is necessary in non-reproductive periods if she is to supply her young with adequate amounts of this substance (28). Macy (29), Sure (27), Tisdall (30) and others have emphasized the dearth of vitamin B1 in the average human dietary, especially as it is commonly prepared (30), and Macy emphasized the lessened susceptibility to infections which followed the feeding of liberal quantities of this substance to the young. In Japan, acute purulent myositis is very frequently associated with human beri-beri. Of 30 cases of myositis 26 were also suffering from beri-beri according to Osawa's (31) report.

A duodenitis which may be present during the first three weeks of the disease was the only lesion that Manson (32) considered specific in acute beri-beri. In beri-beric infants he also found subacute meningitis. Holst (33) also regularly demonstrated duodeno-enteritis in the postmortem examinations of patients dead from acute beri-beri, and sometimes inflammation of the stomach was evident as well.

A great many investigators (34), chiefly Japanese, believe that beri-beri is caused by a specific micro-organism, and that the alimentary avitaminosis renders the individual susceptible to the infection. Manson (32) states that beri-beri is apparently contagious, and suggests that it may have an infectious as well as a dietary cause.

Seventy-three per cent of a group of albino rats whose diets were deficient in vitamin B1 developed microscopic ulcers in the gastric mucosa, according to Dalldorf and Kellogg (35). About 10 per cent of the lesions were chronic and indurated in character, resembling those found in man, as was also their usual site along the lesser curvature. The size of the ulcers in man and the rat are comparable if allowance is made for the differences in the magnitude of the organs.

5. *Occurrence of spontaneous infections in vitamin B2 deficiency*

When rats are almost completely deprived of vitamin B2, they show more marked abnormalities of the eyes, nose, mouth, alimentary tract, and perhaps kidney than of the skin, whereas with a less complete deprivation, which allows the animal to live longer, the skin lesions predominate (36). Whether these lesions in the rat are the counterpart of pellagra in man has been very seriously questioned recently (37, 38). However, a dermatitis sometimes accompanied by oedema and always followed by desquamation and loss of hair; adherent eyelids with varying degrees of conjuntivitis; fissures, ulcers and soreness about the mouth; inflammation and small ulcers of the tongue, and occasionally crinkled or inflamed ears are all or in part found in rats that have had very little or no vitamin B2 for six or more weeks (39). At postmortem examination Thatcher found that the intestinal tract was very unhealthy (39). Findlay (39) reported that the intestines showed intense congestion of the submucous layer with atrophy of the lymphoid and muscle tissues, and Chick and Roscoe (39) described their findings as an inflammation and atrophy of the mucous membrane. The latter was also often covered with blood stained mucus. Whether these lesions are partially due to infections is at present uncertain, but the congestion of the blood vessels, the infiltration with polymorphs and round cells, and the changes in the adrenal which resembled those caused by slight amounts of toxins which Findlay's (39) study revealed, would suggest that such was the case. On the other hand, the symmetrical distribution of the lesions does not fit in with this theory. Salmon, Hays and Guerrant (40) state that they isolated a Gram-positive coccus, often in pure culture, from skin lesions, arthritic lesions, parenchymatous organs and from the walls of the intestines in vitamin B2 deficient rats. The organism was recovered only twice from the blood. If heavy growths of this coccus were fed to rats, characteristic lesions from which the organism could be recovered were produced. Up to the present however the author of this review has seen no confirmation of these results.

That cataract is a common occurrence in rats fed diets lacking in vitamin B2 has recently been reported by Day, Langston and O'Brien (41). Anterior interstitial keratitis and conjunctivitis, apparently caused by commonly avirulent organisms, frequently were also present, but there was no evidence that any infection had invaded the eye from the outside. Iritis and cyclitis were not found and the authors incline to the opinion that the cataracts were due to some nutritional change. Jobling and Arnold (42) have collected data in support of their theory that the deficient diet in pellagra allows the overgrowth of a fungus which is, by means of the photosensitization which it produces, the indirect cause of the dermatitis. In other words, the dietary inadequacy lowers the body's resistance to the fungus.

6. Susceptibility to artificially induced infections

(*a*) *Reduced resistance in vitamin B deficient animals.* In 1922 and 1923, Findlay (5, 15) reported that pigeons which had been kept on a vitamin B deficient diet for 25 days or more died in large numbers after intraperitoneal injections of pneumococci, meningococci, B. coli, or B. enteritidis (Gärtner), whereas adequately fed controls died only occasionally. If the birds had been fed the inadequate diet for only 15 to 20 days, their resistance was apparently not lowered, and the drop in the resistance seemed to be coincident with the fall in the body temperature.

These findings were confirmed by Werkman (43) who found that 80 per cent of pigeons deprived of vitamin B died after the intraperitoneal injection of B. anthracis, whereas none of the normal controls succumbed.

The rectal temperatures were abnormally low in these avitaminic birds. Both the pigeon and the rat are naturally immune to anthrax. Rats were used in the later experiments and the results were similar. The same author (14), in collaboration with Baldwin and Nelson, reported that 28 units of diphtheria toxin per 10 grams weight was sufficient to kill a vitamin B deficient rat, whereas twice or almost three times this amount was needed if the animal was fed a complete diet. Blackberg (10) found that such was also the case when tetanus toxin was used. In his experiments the deficient rats succumbed to doses 40 to 100 times less than those fatal to the normal controls.

Guerrini (8) and also Biondo (1) stated that pigeons lacking vitamin B were no longer immune to anthrax, and Setti (9) confirmed the increased susceptibility of such birds to infections.

In 1923, Bassett-Smith and Gloyne (44) compared the duration of life, after subcutaneous inoculation with tubercle bacilli, of guinea pigs fed very little vitamin B and others fed large amounts of this substance. The first experiment, which included four animals, gave inconclusive results. In the second experiment a smaller dose of bacteria was used and the three animals fed the generous allowances of vitamin B survived longer than the one deficient animal. As individual guinea pigs vary in their reactions to tubercle infection, these small differences are probably not significant.

McCarrison (45) reported that when wild monkeys were placed on a diet lacking vitamin B, colitis, probably due to Entameba histolytica, developed in about 40 per cent of them. The disease did not appear in controls fed a normal diet, whereas in a third group that lacked vitamin A as well as vitamin B, all were afflicted.

Rowlands (46) put rats on diets partially and completely deficient in vitamin B alternately for twelve weeks. He then fed them 1 cc. of a heavy suspension of streptococci or other organisms. The controls that had received bemax (a source of vitamin B) in addition to the diet were similarly infected. The infection did not cause any apparent disease in the rats, and after four days they were killed. Sections of the intestinal walls of the deficient animals that had been fed streptococci showed these organisms in the lacteals, and they were also present in the intestinal lumen in large numbers. The controls showed only a few streptococci in the lumen and none in the lacteals. The experiments however would have been much more convincing if an actual infection had been set up.

In one of Rose's (47) experiments on a dog deficient in vitamin B, an abscess yielding a pure culture of the Welch bacillus (B. aerogenes capsulatus) developed at the site of a previous subcutaneous injection of vitamin B solution. The dog recovered rapidly when it was fed large amounts of vitamin B. Dogs on a normal diet could not be infected with this bacillus, whereas those on rations deficient in vitamin B developed positive blood cultures after infection. These cultures became sterile when the animals were fed vitamin B.

About 19 per cent of Hagedorn's (48) rats that were fed a diet very poor in vitamin B recovered from a bovine tuberculosis infection introduced intraperitoneally. On the other hand, 54 per cent of the rats fed the same diet with the addition of yeast survived.

Lassen (49) fed twenty mice deficient in vitamin B and nineteen controls 0.5 cc. of B. aertrycke (Breslau) broth. Some of each group were killed daily to determine if the infection was disseminated more rapidly in the deficiently fed animals, but no difference in the rate of spread was revealed. All of the remaining animals died. The mice deficient in vitamin B died on the average in eight days and the controls lived slightly longer, nine and six-tenths days. This difference is probably negligible. The mouse is very susceptible to this infection, and the large dose may have masked any difference in the resistance between the two groups.

Rats on similar diets were also used by this same author. After three and one-half weeks on the diets the animals were infected as in the previous experiment and later killed and examined. In the first experiment none of the animals died, but the deficient rats showed slightly more severe infections. In the second experiment the four deficiently fed animals died with marked infections, and none of the controls did so. Three non-infected B deficient rats were also followed and they did not die until after the conclusion of the experiment. In all, seventeen rats were infected in the two tests.

When Pfannenstiel and Scharlau (50) infected rabbits intracutaneously with hemolytic staphylococci, they found that marked inflammatory reactions resulted, which took about a month to heal. If small doses of irradiated ergosterol were given, the reaction was smaller and healed in two weeks. When vitamin B, as well as vitamin D, was fed, the size of the lesion was still smaller and the rate of healing was speeded up.

Ackert and Nolf (51) have shown that young chickens deprived of vitamin B cannot recover from an artificial infection with ascarid worms as well as controls fed the same diet plus 6 per cent yeast.

Puppies kept on a diet lacking in vitamin B until their weight began to decline, and others on a complete diet were infected both by mouth and through the skin with mature larvae of the dog hookworm by Nagoya (52). By a very laborious process the number of larvae present in the tissues after maceration was counted and it was found that there were almost twice as many in the deficient as in the control animals after either method of inoculation. These results were confirmed by means of serial sections in a later test. In other experiments he fed much smaller doses of larvae and then compared the percentages of the worms that became mature. It was found that 35 per cent reached maturity in the vitamin B deficient dogs and only 23 per cent in the dogs fed adequate diets. In other words, more of the larvae survived and more reached maturity in the dogs that lacked vitamin B.

In 1932, the author, in collaboration with Ross (53), tested the resistance of vitamin B deficient and normal rats to oral infections with S. enteritidis. The rats were infected one to eight days after they were put on the diets and practically all of the deaths occurred within 24 days after the commencement of the diet. The animals were therefore infected in the early stages of the avitaminosis. An almost equal number of litter mates were kept on the rations without being infected and none of these deficient animals died from the effects of the diet alone before the termination of the experiments. The dead rats showed typical changes due to the infection and the infecting organism was regularly recovered from the heart's blood. The total results showed that only 19 per cent of 53 animals on the vitamin B deficient diet survived in contrast to 75 per cent of 51 animals on the complete diet.

In 1933, Kobashi (54) was able to set up a purulent submaxillary adenitis in rats by feeding them a coccobacillus which he had isolated from a similar spontaneous infection. He found that the administration of vitamin B reduced the severity of the disease to some extent.

This vitamin also ameliorated the course of the infection that followed the injection of staphylococci into the skin of rats.

(*b*) *No reduced resistance in vitamin B deficient rats.* In Hotta's (55) experiments small groups of mice fed a complete diet and others fed the same diet with the omission of vitamin B were infected intraperitoneally with four different doses of mouse typhoid bacilli. No difference in the precentage of survivors was shown between the avitaminic and the normal mice.

Verder (56) made use of rats fed either a normal diet throughout or vitamin B deficient and normal diets alternately. In the latter animals the deficient diet was given for the last two weeks before infection. The rats were then fed suspensions of enteritidis bacilli and killed, examined and cultured at short intervals up to 48 hours after the infection. Except for the persistence of the bacilli in the duodenum and jejunum of about half of the deficient animals, the results were negative. One positive spleen culture was obtained but it was from a normal rat. The reason for the use of alternating complete and deficient diets is obscure and the animals were also probably killed too soon after the infections.

Reiner and Paton (57) have recently reported some surprising results. Rats fed adequate and vitamin B complex deficient diets were infected with a trypanosome. The former rats died regularly in about four and one-half days, whereas the latter died about a day later on the average, although occasional rats lived three to five days longer. They all ultimately died. A lack of vitamin B1 alone had almost as great an effect, and the absence of vitamin B2 possibly produced a similar, but much less marked delay in the onset of death. Several attempts to discover the explanation of the phenomenon yielded no results.

Summary. A great variety of experimental infections have been shown to be more frequently fatal or more easily induced in animals lacking vitamin B than in normal animals. Very few reports have been published which have not shown this reduced resistance. Of these, Hotta's experiment was carried out on comparatively few animals and was not repeated, and Verder's rats were killed too soon after infection. Her method also was planned to reveal the course of the infection rather than the degree of resistance of the animals. The difference in the time of survival after inoculation with trypanosomes which Reiner and Paton described was small.

Comparatively few of the investigators infected the animals when they were in the early stages of the deficiency and more of such experiments are needed. It is not surprising that an animal suffering from marked deficiency succumbs when an infection is superimposed. The finding that early deficiency lowers the resistance would be of more practical importance. The effect of partially deficient diets has not been investigated, nor has that of vitamin B1 or B2 separately except by Reiner.

Several investigators have found that the administration of vitamin B helps an animal to overcome an infection that has already become established (Rose, Pfannenstiel and Kobashi). Lassen and also Rowlands have found evidence that after oral infection bacteria are disseminated more extensively in vitamin B deficient than in normal rats.

7. *The use of vitamin B in clinical infections*

In the last few years several authors (58) have stated that when more vitamin B is added to the usual diets of infants and children increased weight gains result. One would therefore infer that the original diets were somewhat lacking in this factor. In addition, other investigators (59) have remarked on the decreased susceptibility to upper respiratory and other infections which accompanied this increased gain in weight. Sure (27) and others have emphasized the fact that the amount of vitamin B in present day American diets is low, but whether some of the cases of chronic ill-health and obscure gastro-intestinal and cardiac disorders are the outcome of this deficiency (60) is at present speculative.

Many clinical reports on the value of yeast in the treatment of furunculosis have been published (61). Whether this good effect is due to the high vitamin B content of the yeast, or to its effect in altering the intestinal bacterial flora and decreasing putrefaction, is as yet unknown. That the vitamin B in the yeast might improve the patient's reaction to the infection is certainly possible. Cowgill and his co-workers (19) suggest that decubital ulcers may be due to the lack of vitamin B. Yeast has also been found by a number of authors to be helpful in cases of acne (61). The results have however not been uniformly good as a much larger percentage of Hawk's cases were cured than of Reeve's or Welker's. The same authors and others have found yeast to be beneficial in the treatment of constipation (61, 62). As vitamin B decreases intestinal stasis by improving the activity of the intestinal musculature, the phenomena which are responsible for this good effect are complicated and infection probably does not play any direct part.

Rowlands found that cases of bacilluria cleared up when generous amounts of vitamin B were fed (46).

Fletcher (63) and also Rowlands (46) advise increasing the vitamin B intake very considerably in cases of chronic arthritis. The foci of infection are cleared up whenever possible also, and the latter author uses vaccines as well. In such cases, the large intestines are usually distended, with resultant delay in the emptying of the bowl. Under vitamin B treatment the intestinal abnormalities generally disappear, and in addition the absorption of toxins and of bacteria is probably reduced. The vitamin B may also aid the individual to overcome the infections that are often found in such patients.

In 1923, Gerstenberger (64) treated a series of patients suffering from herpetic stomatitis and herpes labialis with yeast vitamin tablets (vitamin B concentrate) and found that these conditions, even when they had advanced to the stage of ulceration, were rapidly cleared up by this means. In about one-half of the cases Vincent's organisms were present. This author believed that the bacterial infection was secondary to a disturbance in metabolism resulting from an insufficient intake of vitamin B. Such a disturbance of metabolism might be described in other words as a state of decreased resistance of the tissues to bacterial injury. Lardier (65), in 1902, advocated yeast in the treatment of pneumonia and bronchitis.

Whether vitamin B is of particular value in the treatment of tuberculosis is at present an open question. In 1926, Lecoq and Fournier (66) found that the administration of a yeast extract with the addition of a small amount of manganese improved the general condition and also the course of the disease in a few patients with pulmonary tuberculosis. Bray (26) also stated that yeast was helpful in the treatment of tuberculosis. However, until this effect has been observed in many more individuals, judgment must be suspended as to the value of such treatment. More recently, Scheurlen and Orlowitsch-Wolk (67) have reported that the administration of extra vitamin D and vitamin B to unselected sanitorium cases of tuberculosis does not promote progress in any way. They had previously shown that additions of vitamin D alone had no effect in similar cases.

Shastid (68), in 1929, found that two cases of optic neuritis of unknown origin responded quickly and completely to vitamin B therapy.

Summary. Whether an increased intake of vitamin B will reduce the incidence of respiratory infections in the young, or of chronic ill-health in older individuals is at present undecided. The fact that this complex, and particularly the B1 fraction, is consumed in relatively small amounts by many individuals would suggest that the effect of its addition should be tried out on a large scale. Yeast, which contains large amounts of these vitamins, is apparently of value in furunculosis and probably in acne also. Many cases of chronic arthritis seem also to be very definitely benefited by increasing the vitamin B intake. Gerstenberger's very successful results with cases of herpetic stomatitis and herpes labialis have not yet, as far as the present author is aware, been confirmed. The experiments in which the effect of these factors on tuberculosis have been tested are few in number and have yielded conflicting results.

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VITAMIN C

1. Variations in the natural immune bodies or tissue reactions in vitamin C deficiency

(*a*) *Results indicating that these are reduced.* Fortenato (1) reported in 1921 that the opsonic index was lower in scorbutic than in normal guinea pigs. In the following year, Leichentritt and Zielaskowski (2) measured the trypanocidal substance in the blood of guinea pigs suffering with scurvy and found that it was reduced. Höjer (3) however criticized the latter's experiments on the grounds that they were carried out on too few animals.

According to Prausnitz and Schilf (4) tuberculous scorbutic guinea pigs show considerably smaller tuberculin reactions, which also disappear more quickly than those in tuberculous guinea pigs subsisting on normal diets. The febrile reaction after the tuberculin injection was also less marked in the scorbutic animals. This reduced skin reactivity was not correlated with a generalized unsusceptibility to tuberculin (5) as the animals with scurvy died more frequently than the normal controls when this substance was injected subcutaneously in large amounts (5 cc.).

In addition, Bieling (6) and also Arkwright and Zilva (7) found that markedly scorbutic guinea pigs gave smaller skin reactions to diphtheria toxin than normal. The former author noted that the necrosis of the skin was slower coming on, and that the subcutaneous oedema was absent or very slight. The latter authors reported that animals on diets which contained suboptimal amounts of vitamin C, but enough to allow a gain in weight of about 25 per cent, still showed large Schick reactions, whereas if this vitamin was further reduced so that a loss of about the same magnitude occurred, the reactions were very small. Scorbutic guinea pigs however are definitely more susceptible to large doses of diphtheria toxin and die earlier than normal animals according to Bieling. A possible clinical application of these findings was provided by Hess (8) in 1932. He had encountered nasal diphtheria very commonly in children with scurvy. The Schick reactions were regularly negative, although the patients showed the bloody mucous nasal discharge which is typical of this disease, and one child apparently died from it. In three cases, virulence tests showed the bacilli to be virulent. The last of these three cases gave no skin reaction to dilutions of from $\frac{1}{50}$ to $\frac{1}{5}$ M.L.D. of toxin. In his brief review the author does not discuss the possibility of these cases being carriers, already self-immunized. He suggests that in scurvy the pharyngeal mucous membrane loses its immunity to the diphtheria bacilli, whereas the general immunity as reflected by the negative Schick test is still maintained. A simpler explanation however might be that the scorbutic skin does not react in the usual manner to the toxin, although the organism as a whole is not immune to it.

Lawrynowicz (9) suggests that scurvy may so reduce the resistance that a carrier may become the victim of bacteria which it previously carried with impunity. For example, a guinea pig that had been well for one month after it had been used in a crude test for B. diphtheria was placed on a scorbutic diet. Thirty-seven days later it died. The post-mortem showed the changes found in diphtheritic deaths and the organism was recovered from the spleen.

When Vercellana (10) injected strychnine nitrate or aqueous extracts of poisonous fungi subcutaneously into scorbutic guinea pigs, he found that they were killed more frequently by these substances than controls fed normal diets. The ration of the deficient animals consisted of oats exclusively. Also aleuronat, broth, peptone, cinnabar and other substances, when injected by Dluzewski (11) into the peritoneal cavities of scorbutic animals, did not provoke the normal inflammatory reaction with the outpouring of leucocytes.

(*b*) *Results indicating that these are not reduced.* In contrast to some of the above findings, Lawrynowicz and Bohdanowicz (9) state that they have never established any difference between the Schick reactions of normal and scorbutic guinea pigs.

In 1919, Zilva (12) determined the complement titres in normal and scorbutic guinea pigs and found that they were the same. Four years later, Hamburger and Goldschmidt (13) reported that the complement titres were not lowered in scorbutic children and guinea pigs. In fact, some of the latter animals showed increased complement titres, which were apparently correlated with high albumin concentrations in the serum. Koch and Smith (14) found consistently increased complement titres in a series of twelve scorbutic guinea pigs. When an antiscorbutic was added to the diet, the titres fell, but still remained somewhat higher than they had been before the onset of the scurvy. On the other hand, Bohdanowicz and Lawrynowicz (9) found that complement did not show any constant or characteristic changes in guinea pig scurvy.

The phagocytic indices in scorbutic guinea pigs were reported by Werkman et al. (15) to be unaltered.

Hamburger and Goldschmidt (13) also determined the bactericidal titres of the sera of scorbutic and normal guinea pigs and of scorbutic and normal children to the same strain of colon bacillus and found that they were similar. This organism was used because the pyelonephritis which frequently complicates guinea pig scurvy is usually caused by it.

2. Variations in acquired immune bodies due to vitamin C deficiency

(*a*) *Results indicating that these immune bodies are altered.* When scorbutic guinea pigs were sensitized to horse serum, or red blood corpuscles, Zolog (16) found that they were much less sensitive to anaphylactic shock than normal diet controls. The minimum lethal dose was three to ten times higher in the animals with scurvy. Sereni (17), on the other hand, reported that scorbutic guinea pigs showed much more severe anaphylactic shock than the control animals. Hurwitz and Wessels (18) went further into the question and found that the uterine muscles of sensitized vitamin C deficient guinea pigs would not react either to the specific antigen or to smooth muscle stimulants, whereas the bronchial muscles of such animals reacted normally. In addition, when Bieling (5) immunized scorbutic guinea pigs with diphtheria toxin, he found that they did not produce as much antitoxin as the adequately fed controls.

(*b*) *Results indicating that these immune bodies are not reduced.* Scorbutic and normal guinea pigs produced agglutinins to B. typhosus equally well according to both Zilva (12) and Werkman (15). In addition, the former author stated that amboceptors to the same organism were also produced in normal amounts by guinea pigs on vitamin C deficient diets, and the same findings also held true for the rat. In 1922, Hess (19) reported that the diphtheria antitoxin production in scorbutic guinea pigs was as good as that in normal controls.

Summary of immunological investigations. I. Non-immune animals. In several of these studies conflicting results have been obtained. For example, Werkman reported that the opsonic indices of non-immune scorbutic guinea pigs were as high as those of normal animals, whereas Fortenato found them reduced. And again, Lawrynowicz stated that the presence or absence of scurvy did not affect the size of the Schick reaction in guinea pigs, whereas Bieling and also Arkwright found these reactions considerably reduced when scurvy was present. Other workers reported that tuberculin reactions were also considerably decreased. As the immunological significance of the Schick and tuberculin reactions are entirely different, one would infer that the general reactivity of scorbutic skin was depressed. The smaller Schick reactions were not due to any increased antitoxin in the animal, as Bieling showed that these guinea pigs died more frequently and more quickly after the injection of large amounts of toxin. In fact, scorbutic guinea pigs seem more susceptible to the subcutaneous injections of toxic substances generally, e.g., to tuberculin, strychnine and poisonous fungus extract. Lawrynowicz suggests, on evidence gathered from the study of one animal only, that scurvy so lowers the resistance of a healthy carrier that it may become the prey of bacteria which formerly did not harm it. This sequence of events however might have occurred without the aid of the scurvy-producing diet. Leichentritt found that the substance in the blood which destroyed trypanosomes was reduced in scurvy, and further evidence of the reduced capacity of the scorbutic animal to cope with infections was provided by Dluzewski, who reported that the inflammatory reactions which followed the injection of foreign substances into the peritoneum were much reduced. Two authors stated that the complement titre was unchanged in scurvy, but a similar number of investigators found it increased. One of the latter however did not find it consistently raised, but at least it was never lowered.

II. Immune animals. Comparatively few studies have been carried out on such animals, and many of the results are conflicting.

For instance, Hess found that scorbutic guinea pigs could produce diphtheria antitoxin as well as normal animals, whereas Bieling states that this is not the case. Zilva and Werkman were not able to demonstrate any difference between the amounts of anti-typhoid antibodies produced by guinea pigs and rats lacking vitamin C and those fed adequate diets.

The results of the anaphylaxis experiments are of interest because most of them suggest a reduced activity in the tissues of animals suffering from scurvy, analogous to the lessened skin reactions.

3. *Occurrence of spontaneous infections in vitamin C deficiency*

(*a*) *Infections indicating a reduced resistance. I. Experimental.* In 1932, Suzuki (20) stated that the nasal mucous membrane and glands were atrophied and showed catarrhal inflammation in vitamin C deficient guinea pigs. The crushed oats, autoclaved milk diet that McCarrison (21) fed his guinea pigs is mainly lacking in vitamin C. He found that the bladders in such animals at postmortem examination were tightly contracted and that the mucous membrane of this organ was congested and necrotic. The duodenum was also intensely congested and punched out ulcers were present in the intestines and sometimes in the stomach. Mackie and Chitre (22) gave their monkeys very small amounts of orange juice, but most of them developed scurvy, and in addition they showed in their large intestines very marked necrotic and ulcerated lesions, which were laden with common intestinal bacteria. These various pathological findings provide possible explanations for some of the frequent secondary infections that occur in cases of human scurvy.

In Höjer's (3) series only about 30 per cent of his severely scorbutic guinea pigs showed infections. This low figure may be partly explained by the fact that they survived for just a few weeks. On the other hand, 50 per cent of the animals with mild scurvy developed infectious lesions, and about 20 per cent of the much longer-lived normal animals showed similar lesions.

In the course of his experiments, Heymann (23) reported that he lost a large number of scorbutic guinea pigs with pneumococcic pneumonia.

II. Clinical—latent scurvy. Even before the onset of definite symptoms of human scurvy, in the so-called period of latent scurvy, the affected individual is particulaly susceptible to infections (24) and if these are contracted they run an unusually severe course.

In 1919, Wiltshire (25) described the occurrence of small conical swellings in the hair follicles of the legs of scorbutic Serbian troops and he also found them during the scurvy season (January and June) in apparently normal individuals. The latter were probably suffering from latent scurvy.

One of the most typical pathological lesions in scurvy is the increased permeability of the blood vessel wall which allows the blood to ooze into the tissues. Göthlin (26) was able to devise a method of measuring the permeability of the cutaneous capillaries. In 1931, he found that 18 per cent of a group of apparently healthy Swedish country school children (11 to 14 years) were suffering from vitamin C undernourishment. Hopkins (27) was able to associate a period of ill health in boys in a preparatory school with a lack of fresh fruit and vegetables during the winter months. When a little fresh fruit was supplied, the minor ailments and the listlessness disappeared.

In children who are suffering from undiagnosed latent scurvy, vaccination may precipitate acute scorbutic symptoms (28, 29). Abels (29) quotes the case of an anemic, atrophic ten months old child who developed both scurvy and a high prolonged fever after vaccination. This may explain the reluctance of parents in backward regions of Austria towards having their children vaccinated in the winter, when no doubt their diets are partially deficient in this vitamin. In such children, coryza and pharyngitis may be surprisingly severe and may usher in evident scurvy, and skin ulcers and cystitis are also very prevalent. In fact, this author has gone so far as to say that manifest scurvy is always preceded by an infection. Other investigators (30) however have found this sequence of events to occur frequently, but not invariably. The increased metabolism caused by the infection probably accentuates the vitamin deficiency and hastens the appearance of active scurvy.

As in the case of the other deficiency diseases, there seems to be some predisposition to scurvy, as only a certain number of those on a uniformly deficient diet develop it (24b).

Manifest scurvy. Infections are very commonly associated with active scurvy (31), and Von Niedner (31) reported that scorbutic soldiers succumb to the slightest infection. Numerous authors (29, 32) have found respiratory infections, including grippe and pneumonia, to be very common in such individuals. One of these authors, Erdheim (33), stated that such diseases were frequently very grave and persistent in scorbutic children. Tuberculosis was also very prevalent in several series (32b, 34). In one of these, Salle and Rosenberg (34) found that all the deaths (17) in their 461 cases were from tuberculosis and that 9 to 22 per cent of their different groups of scorbutic patients suffered from this disease. They also remarked on the great frequency with which cases of infantile scurvy were complicated by florid tuberculosis. Diphtheria (8, 32b, 34b) and dysentery and typhoid (29, 34a, 35) were also very often encountered by various clinicians in scorbutic individuals. Mackie (22) described an epidemic of dysentery (Shiga) among scorbutic war refugees in the near East, which was almost as virulent as cholera. Many investigators (32b, 35, 36) have reported that cystopyelitis and nephritis were very common, and that furuncles, paronychia and gun shot wounds (2, 32b, 35, 36) were often very difficult to clear up in scorbutic patients.

In 1927, Funk (37) stated that an epidemic of pneumonia in the Sudan disappeared when antiscorbutic treatment was given to the numerous cases of scurvy which appeared at about the same time. This would suggest that scurvy lowered the resistance to this infection.

Oral infections. If a guinea pig is kept on a completely vitamin C free diet for even two days, marked abnormalities are seen in its teeth (3, 30), and if such a diet is kept up for a few weeks, the teeth may become devitalized. Apical abscesses are prone to appear in such teeth later on. The same processes may occur in man (38), and the resistance to infection may be indirectly lowered by the presence of these bacterial foci. Höjer and Westin (30) also found that although enough vitamin C was given (1.2 minimum protective doses of orange juice) to prevent the appearance of any scorbutic changes in the teeth, except perhaps an uncertain hyperemia in the pulp cavity, the animals were still markedly susceptible to infection.

After analyzing the diets of groups of individuals, Hanke (39) stated that those whose diets were complete suffered from dental caries, gingival irritation or pyorrhoea much less frequently than those whose diets were deficient in either or both vitamin C and vitamin D. The details of the diets were unfortunately not given. Spongy gums, associated with infections, were cleared up by the use of an adequate diet plus 1 pint of orange juice, the juice of a lemon and from one-fourth to one-half a head of lettuce daily. The resistance to other infections, especially to colds, was raised at the same time, and in one individual a long standing osteo-myelitis was also cured. When pyorrhoea was present surgical measures had usually to be combined with the dietetic treatment unless the condition was very mild.

4. *Susceptibility to artificially induced infections*

(*a*) *Reduced resistance in vitamin C deficient animals.* In 1923, Findlay (40) reported that guinea pigs fed on a vimamin C deficient diet died more frequently after intraperitoneal injections of bacteria than controls fed on normal diets. The organisms used were B. coli, staphylococcus aureus, streptococcus hemolyticus and pneumococcus.

In the same year, Werkman and his co-workers (15) found that there was a definitely, although not markedly, increased susceptibility to intraperitoneal injections of pneumococci or B. anthracis in scorbutic guinea pigs as compared with controls.

According to Abels (41), guinea pigs with scurvy die after intraperitoneal injection of B. coli, whereas normal animals withstand several times this dose.

B. aertrycke cultures were fed to 2 scorbutic and 2 normal guinea pigs by Grant (42). One of the scorbutic animals died and the three others were killed so that the spread of the bacilli to the various organs and the blood could be determined. Liver, spleen, lung and blood cultures were negative in the normal animals, whereas both the spleen and one of the blood and one of the liver cultures from the scorbutic animals yielded B. aertrycke. These findings would suggest that in scurvy the intestinal wall is more permeable to bacteria.

Schmidt-Weyland and Költzsch (43) infected normal and scorbutic guinea pigs by either inhalation or feeding, or by the combination of both methods, with a mixture of pneumococci and a fowl cholera pasteurella strain. They found that the animals on the scurvy producing diet were much more susceptible to such infections and that many of them died of pneumonia.

A trypanosome infection was set up in half their scorbutic guinea pigs by Nassau and Scherzer (44). They reported that this procedure hastened the onset of the scurvy, but only slightly decreased the duration of life.

Höjer (3) divided about ninety guinea pigs into several groups which were fed normal, completely vitamin C deficient, and several different partially C deficient diets. Half of each group was infected intramuscularly with probably too large a dose of a low virulent human strain of B. tuberculosis. All of the four severely scorbutic animals showed larger lesions than many of the rest. Only one guinea pig, which was fed the normal diet, showed no evidence of the disease, except for fibrous healing at the site of the subcutaneous injection. The course of the disease did not parallel the degree of scurvy in the partially scorbutic animals, but microscopic examination showed that the connective tissue reaction to the tuberculous foci at a specified time after infection varied directly with the amount of vitamin C in the diet. The more vitamin C fed, the more adequate was the connective tissue response.

Coulard (45) stated that the tuberculous processes at the site of injection, the enlargement of the glands, and the lesions in the spleen developed much more rapidly in the scorbutic than in the normal guinea pig.

Guinea pigs suffering from slight scurvy were reported by Heymann (23) to be no more susceptible to tuberculosis than normal animals. When however the scurvy was moderately severe, marked loss in weight and early death (73 days) followed infection with a human strain of tuberculosis. Similarly infected guinea pigs fed on a normal diet lived 141 days on the average.

In order to induce intestinal tuberculosis in the guinea pig after the feeding of tuberculous sputum, McConkey (46) found that a partial deficiency of vitamins A, C and D was necessary. However, the lack of vitamin C seemed to be especially important.

Bieling (5) was able to produce a localized chronic tuberculosis in his guinea pigs. These animals were strong and well nourished and remained in such condition for over a year. If, however, they were put on a vitamin C free diet, they seemed particularly susceptible to scurvy and died long before the non-infected controls. These early deaths could be attributed to an activation of the chronic tuberculosis by the scurvy, although the sections showed neither very marked scurvy nor tuberculosis extensive or severe enough to explain the rapid deaths. This increased susceptibility of the tuberculous animal to scurvy was gradually built up, as recently infected animals did not react differently from uninfected ones. If the amount of vitamin C in the diet was reduced but not absent, the same phenomena were observed, but the onset of scurvy and the deaths were delayed. Apparently therefore the development of scurvy is accelerated when tuberculosis is present.

Quite a number of studies on this subject have been carried out by Mouriquand and his collaborators. In 1924, they (5b) showed that a larger percentage of scorbutic than of normal guinea pigs died after the injection of tuberculin. In 1925 (47), they determined the effect of the injection of fairly large (10 million) and very small numbers (400) of tubercle bacilli into chronic scorbutic and normal guinea pigs. When the massive dose was used, for the first three weeks the deficient animals showed less extensive lesions and less loss in weight than the controls. After this time the scorbutic animals went rapidly down hill and died before the controls. With the smaller dose no initial refractory stage was seen, and the lesions in the animals with scurvy progressed more rapidly and led to earlier death. Two years later, they reported that if after feeding a diet completely deficient in vitamin C, a ration partially lacking in this factor was given, a chronic scurvy was established which was characterized by a tendency to relapses of the active scurvy, and by great susceptibility to infection with B. tuberculosis. When such an infection was set up, the animals suffering from chronic scurvy lost weight and died after a short time, and there was not the slightest evidence of tissue reaction against the bacilli, even though these were much attenuated. Normal animals similarly infected reacted with "multiple" sclerosis and lived considerably longer.

(*b*) *Increased resistance due to the addition of vitamin C.* The addition of vitamin C rich lemon juice to an adequate diet favorably influenced the course of tuberculosis in guinea pigs, according to Leichentritt (48). The experiments of Héricourt and Richet (49) may possibly be interpreted as providing further confirmation of the important rôle played by vitamin C in this disease. They found that if dogs were injected with raw meat juice they withstood a tuberculous infection better than similar animals injected with cooked meat juice. The cooking no doubt destroyed the vitamin C, but it may have had other deleterious effects on the meat juice as well. When the diet contained vitamin D, Grant (50) found that increasing the amount of vitamin C seemed to decrease the severity and extent of the tuberculous lesions in the lungs of guinea pigs.

(*c*) *No reduced resistance in vitamin C deficient animals.* In some of Grant's (50) other experiments she used diets in which the vitamins were unbalanced and the results were entirely different. For example, she reported that if vitamin D was deficient in the diet, the addition of vitamin C tended to increase the amount of tuberculosis in the lungs, and the same effect also followed the substitution of vitamin C for vitamin D at the time of inoculation.

In one of their earlier publications (1922), Mouriquand (51) and his co-workers reported that chronic scurvy did not accelerate the course of tuberculosis in the guinea pig. Their later work gave results entirely opposed to those of this early investigation.

Bieling (5a) stated that "transitory milk or hunger scurvy" did not lead to a decreased resistance to infection.

When Jaffe (52) infected the leg bones, muscles or skin with staphylococci and put the guinea pigs on a scorbutogenic diet at the same time, he found that about half of them developed severe infections and that these animals lived longer (42 days) than the uninfected controls, and did not show scorbutic changes at death. If the infections were mild, death from scurvy occurred at about the usual time (21 to 30 days). If the animals were on the deficient diet for 10 days before infection, they died abnormally quickly from the scurvy (7 to 12 days). Baj (53) partially confirmed these findings when he reported that the characteristic bone changes of scurvy were less marked in animals infected with staphylococci. He suggested that antiscorbutic substances were formed by the bacteria. He also stated that the infections in scorbutic animals were no more severe than those in controls fed normal diets.

As many mice on a vitamin C deficient diet survived after intraperitoneal injections of mouse typhoid bacilli as mice on a complete diet, according to Hotta's (54) results.

Summary of artificial infection experiments. Relatively few of these investigators have brought forward evidence to the effect that a deficiency of vitamin C does not lead to a lower resistance to infection, and some criticism of their work is possible. For example, Hotta's results were based on one experiment including at the most 32 rats, and the rat is apparently able to synthesize this vitamin, and Mouriquand's numerous later results contradicted his earlier report, which need not therefore be considered further.

On the other hand, Findlay, Werkman and also Nassau found that a greater proportion of scorbutic than of normal guinea pigs died after intraperitoneal injections of bacteria or trypanosomes. The last two authors stated that the reduction in the resistance was not marked. Jaffe infected the legs of guinea pigs that had been on a scurvy producing diet for ten days with staphylococci and found that they died very quickly. As Schmidt-Weyland's method of infection more nearly simulates that occurring in nature, it is probably preferable to those used by the above mentioned authors. Schmidt-Weyland's results showed many more deaths from pneumonia among the scorbutic animals.

The interest in the question of whether scurvy renders an animal particularly susceptible to tuberculosis was possibly engendered by clinical reports to that effect. The guinea pig develops scurvy readily and it is also very susceptible to tuberculosis. It is probably more susceptible to both these conditions than man. Consequently, in most of these experiments the resistance has had to be gauged either by variations in the duration of life or in the extent and nature of the lesions. As the course of tuberculosis in even normal guinea pigs is variable, these criteria are somewhat unsatisfactory. According to Heymann, the susceptibility varies with the severity of the scurvy. Slight scurvy does not affect the resistance, whereas animals suffering from moderately severe scurvy are less resistant and die quickly from tuberculosis. Höjer's experiments, which might have confirmed Heymann's, gave variable results from the point of view of duration of life. Coulard and also Mouriquand found that tuberculosis was fatal more quickly in scorbutic than in normal guinea pigs. When Höjer examined his animals in regard to the extent of the lesions, his results were more consistent, as the markedly scorbutic animals showed the greatest involvement, the normal the least, and in the slightly scorbutic the lesions were variable. Coulard also remarked on the more extensive tuberculosis found in scorbutic animals. Mouriquand noted that guinea pigs affected with chronic scurvy were unable to produce the usual connective tissue reaction to tubercle infection. Höjer also reported that the efficiency with which this reaction took place varied directly with the amount of vitamin C in the diet.

Several authors have provided information on the part played by bacteria in precipitating acute scurvy. Bieling found that animals with chronic tuberculosis were very susceptible to scurvy and Nassau also stated that the presence of a trypanosome infection seemed to accelerate the onset of scurvy. Jaffe, on the other hand, found that a marked subcutaneous or osseous infection prevented the onset of scurvy and that a mild infection did not affect the course of this avitaminosis.

However, Jaffe's results may possibly have been due to the production of the vitamin by the bacteria. Baj, who suggested the above explanation, also found that the presence of a staphylococcic infection lessened the severity of the scurvy.

From Grant's experiment it would appear that the intestinal mucous membrane in animals suffering from scurvy is more permeable to bacteria, and McConkey indicates that the intestine in such animals is more susceptible to infection.

Three investigators also have shown that added amounts of vitamin C assist animals on normal diets in their reactions against tuberculosis.

5. *The use of vitamin C in clinical infections*

Numerous reports demonstrating the good effect of vitamin rich diets in clinical tuberculosis have been published, but it is impossible to decide what rôle vitamin C plays in such treatment. Also, one can not be sure that the good results which Höjer (3) obtained when he fed a series of twenty tuberculous children raw blood serum (50 to 100 cc.) daily for four months were due to the vitamin C contained in that substance. In a later experiment, the same author (30) compared the effect of the addition of vitamin C (one orange daily) or of added carbohydrate (a pastry) on sanitorium cases of tuberculosis. The patients were grouped in pairs as closely alike in age, sex, tuberculous involvement, and prognosis as possible. One of each pair received the orange and one the pastry. The sanitorium was in an isolated region where the supply of vegetables and fruit was limited, especially in the three months of the experiment (March, April and May). The highest mortality from this disease also usually occurred in these three months. Of the cases fed the extra vitamin C, 17 showed better, 3 showed similar, and 1 showed worse results than the controls. The cases were examined regularly by expert clinicians, and although the effects were not easy to evaluate, it appeared that the provision of plenty of vitamin C assisted in the healing of the tuberculous lesions. Woringer and Sala (55) advised generous additions of vitamin C to whooping cough cases, for although scurvy is very rare in Strassburg, they saw four cases of whooping cough and scurvy together. McConkey (56) reported that the administration of cod liver oil and tomato juice has a favorable effect on intestinal tuberculosis which was secondary to a pulmonary infection. In order to determine whether the vitamin C was of value he gave three patients on normal diets a cod liver oil concentrate alone. No change could be seen until orange juice was added also, when two of them began to show satisfactory improvement. In a second test, he gave two cases irradiated brewer's yeast. Again they did not improve until the orange juice was administered also. The possibility that the good effects were due to the combination of the vitamins can not be ruled out, as none of the patients were given vitamin C alone. Bloch (57) is of the opinion that vitamin A is of more importance than vitamin C in the treatment of tuberculosis, but other authors (31) claim that generous amounts of vitamin C are essential in the treatment of such cases.

Summary. The results which have been published up to date suggest that this factor plays a very important rôle in the combatting of tuberculous infections, but further investigations will be necessary before this can be conclusively settled.

6. *The mechanism underlying the decreased resistance in scurvy*

According to Höjer (3), the decreased resistance in scurvy is due to the atrophy of the various organs in the body that protect it against infections. These organs include the lymph nodes, spleen and bone marrow. Findlay (40) had previously ascribed the low resistance which he found in scorbutic animals to the changes that were present in the bone marrow.

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VITAMIN E

In 1928, Blackberg (1) reported that rats lacking this vitamin were considerably more susceptible to tetanus toxin than the adequately fed controls. He also found that following the injection of typhoid bacilli the deficient animals did not produce agglutinins or bacteriolysins as well as the controls.

E reference

(1) See Ref. 6 under "Vitamin A and D."

THE POTENTIAL ENERGIES OF OXIDATION-REDUCTION SYSTEMS AND THEIR BIOCHEMICAL SIGNIFICANCE*

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After Harvey's exposition[1] of the circulation a century and a half passed before a chemical description of one of its major functions could be begun. The first step was the isolation of oxygen.[2] Then a period of another century and a half was required for the study of isolated blood components which are involved in the transport of oxygen and carbon dioxide and for the evolution of that theoretical background upon which is sketched a picture of the interplay of systems performing their natural functions. Within the last quarter-century the theoretical treatment has been made so useful that its central theme of adjusting states of equilibria permeates a great part of biochemistry.[3]

One logical progression would seem to be from the mastery of oxygen transport to the study of oxygen use. This study may profit by the previous lessons which teach the following prerequisites: first, the comprehension of components;[4] second, a theoretical framework capable of embracing their interplay. However, the problems here found are soon seen to be so complex that while we may allow their study to originate in the primitive question of oxygen use we cannot let their pursuit be too subservient to the logic and the methods of this particular approach. Only a few specific reasons for this caution need be cited as reminders.

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* Harvey lecture, November 16, 1933.

[1] William Harvey (1578–1657). *Exercitatio anatomica de motu cordis et sanguinis.* 1628.

[2] Joseph Priestley (1733–1804). Dephlogisticated air (O_2) isolated in 1774.
Antoine Laurent Lavoisier (1743–1794). Oxygen named in 1777.

[3] See L. J. Henderson. *Blood. A Study in General Physiology.*

[4] A striking example of the fundamental necessity of a knowledge of components is shown by the revisions of theories of muscle metabolism which are following the isolation of hitherto unknown constituents.

Certain organisms can do without molecular oxygen. They obtain energy by degrading compounds of high energy to those of low. The chemical processes involved seem to be of a type common to the fermenting bacillus and to the working muscle. Also it may be profitable to regard aerobic metabolism as the more complicated because of the integration of oxygen use with primitive processes observable in the isolation of the anaerobic cell. Recently there has been shifted to several hydrolytic processes, such as the hydrolysis of phosphocreatin, a part of the responsibility for immediate action which was originally attributed to oxygen use and then shifted to intramolecular oxidation-reduction. The associations between these distinct types of chemical reactions have made us contemplate anew the coupling of reactions of different classes and the integration of energies of different chemical origins.[5]

These circumstances make room for various researches and they demand of each a theoretical formulation capable of carrying its advance into the description of the whole.

The data which I shall present make comparatively small, concrete contributions to the larger subject. They concern reactions of a type which hitherto has seemed more prominent in laboratory tests than in the living cell; but of this you may judge better after having seen some of the data in review. On the other hand, the data illustrate principles which must have wide bearing and because this theoretical aspect perhaps is the more important I ask you to bear with me while I outline the fundamental concepts of my subject.

Originally the term *oxidation* was restricted to the formation of oxides; *reduction* to the regeneration of a metal from its oxides. However, several and diverse agents as well as oxygen may accomplish the same result; for example, the conversion of ferrous to ferric ion, or of indigo white to indigo blue (by oxygen, chlorine, ceric ion, etc). Generic relations between ferrous oxide and ferrous ion and between ferric oxide and ferric ion are shown in the vertical columns of figure 1. Such generic relations are sufficient for a common classification; but they may not be satisfactory in accounting for the common action of diverse agents in the right $\rightleftharpoons$ left sense of figure 1. A valence change may be expressed as electron[6] exchange and if the process occurs in the presence of water the intervention of its components as molecular, atomic, and ionic hydrogen or oxygen may be postulated. These postulates provide a purely schematic set of interrelationships with the aid of which the common action may be formulated. Since the determinable end-products usually differ in electrons, hydrogen or oxygen, the common action may be crudely summarized, as are the generic relations, in the following definition. *Oxidation* means the addition of oxygen, the withdrawal of hydrogen or the withdrawal of electrons. *Reduction* means the converse process in any case.

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[5] See the discussion by Wurmser in *Oxydations et Réductions*. Paris, 1930.

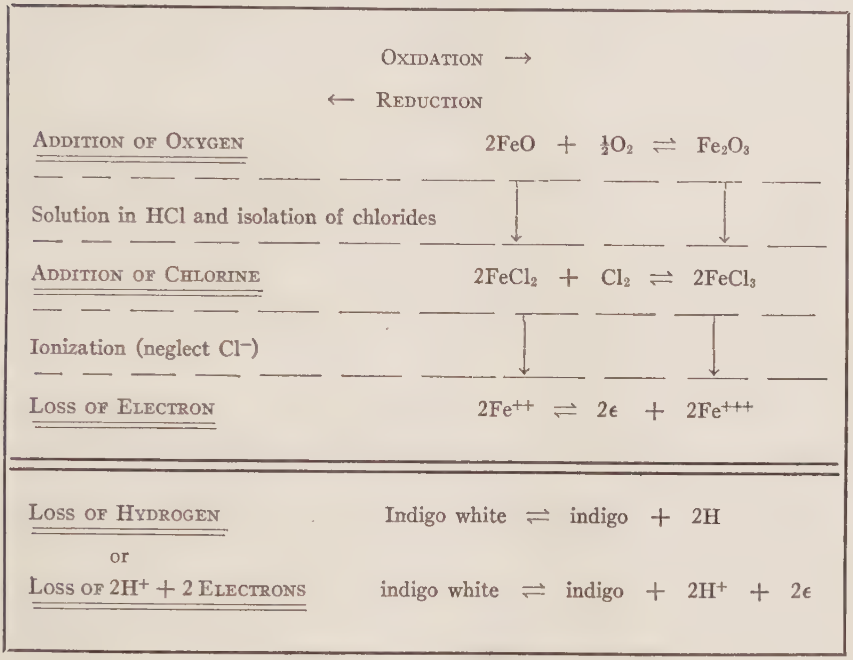
OXIDATION →
← REDUCTION
ADDITION OF OXYGEN
$2FeO + \frac{1}{2}O_2 \rightleftharpoons Fe_2O_3$
Solution in HCl and isolation of chlorides
ADDITION OF CHLORINE
$2FeCl_2 + Cl_2 \rightleftharpoons 2FeCl_3$
Ionization (neglect Cl^-)
LOSS OF ELECTRON
$2Fe^{++} \rightleftharpoons 2\epsilon + 2Fe^{+++}$
LOSS OF HYDROGEN
Indigo white ⇌ indigo + 2H
or
LOSS OF $2H^+$ + 2 ELECTRONS
indigo white ⇌ indigo + $2H^+ + 2\epsilon$

FIG. 1

Between the use of the postulates in arriving at the obvious conclusion of a relationship and the question of which postulates express realities there is a sharp distinction. Failure to maintain this distinction sometimes leads to naïve remarks. For example, as late as 1909 a defender of the chlorine treatment of drinking water declared that this is in no sense a chemical treatment, which the public then considered repugnant, since the function of chlorine is only to release from water nature's bland purifier, oxygen.[7] Convenience has played no small part in making emphasis upon oxygen transport traditional, upon hydrogen transport the choice of biochemists, upon electron transport the choice of analysts.

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[6] The term *electron*, as used here, need only recall the "sign" with which it is associated and the unitary character of electrochemical phenomena with which it is associated. Therefore only a change of expression need be made by those who prefer to regard *the electron* as a hypothetical entity.

However, neither the advantages of a convenience nor evidences for actual participants in specific cases concern us now. We shall have immediate need to elevate the merely formal generalization to a concept which embraces the possibilities among the realities—to the concept of an oxidation-reduction continuum.

A familiar analogy will be illuminating. In the gastric juice hydrated hydrogen ions are doubtless present as discrete particles. Then there is direct significance in the expression "hundredth normal hydrogen ion concentration." Such direct significance is lost when we speak of blood having "four one hundred millionths normal hydrogen ion concentration." This expression is a mechanistic twist of a thermodynamic relation which, more properly translated, might read: "abundant protons, or potential hydrogen ions, act from positions of seclusion within the weak acids of the blood as if supporting this concentration of free hydrogen ions." In very alkaline solution protons may have been stripped from all weak acids, other than water, and the effective agent becomes the hydroxyl ion. A continuum is supported by free hydrogen ions, weak acids, bases, hydroxyl ions. The successful formulation of equilibria among acids, bases and salts is indifferent to a particular mechanistic interpretation. It deals with the acid-base continuum; and, while a pH number may be defined as log $\frac{1}{[H^+]}$, its experimental origin shows it to be a thermodynamic function.[8]

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[7] Example: $Cl_2 + H_2O \rightarrow HOCl + HCl$
$2HOCl \rightarrow 2HCl + O_2$
$O_2 + A \rightarrow AO_2$

[8] The difference of potential between a hydrogen half-cell with hypothetical hydrogen

Later I shall use a technical meaning of the word *potential* but here the word may be applied in the sense which has made it a favorite in the English language. Blackstone says that, when the owner of a property dies or resigns, though there be no actual owner until a successor be appointed, yet there is a legal, potential owner subsisting in contemplation of the law. Both the law of exchange among acids, bases and salts, and the law of exchange in generalized oxidation-reduction contemplate a potential occupant of the field of action as one participant resigns its function to another. Particular chemical structures, environments and concentrations may determine whether electrons or any of the species derived from hydrogen, oxygen or water dominate a particular field of action. Whether or not the student of mechanism gives decision in specific cases there remain uses for the concept of an oxidation-reduction continuum. It will become essential to the description of complex biochemical systems. I shall immediately show a measure of changes in this continuum and it will soon be plain that there is profit in clearing the ground of those too narrow, mechanistic postulates which have tended to divide investigators into opposing groups and which have somewhat of the character which the statesman John Hay called the falsehood of extremes.

A definite measure of a change in this continuum is the energy change, more strictly, the free energy[9] *change.* Since finer distinctions in the

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ion concentration $[H^+]_x$ and the standard half-cell, both with hydrogen at one atmosphere is:

$$-E = \frac{RT}{F} \, ln \, \frac{1}{[H^+]_x}$$

Whence

$$-0.4343 \, \frac{FE}{RT} = \log_{10} \frac{1}{[H^+]_x} = pH$$

The number pH has been of invaluable service in the organization of the subject, acid-base equilibria, especially in graphic presentations. However, it is an unnecessarily complicated function of the energy required to remove one equivalent of protons from whatever state they occupy in a given acid-base continuum and to concentrate them to the one normal concentration of hydrated, hydrogen ions found in the standard. For many purposes it had been better to have organized the subject with the directly measured, hydrogen-electrode potentials themselves. Since they are a linear function of pH they lend themselves as well as pH to graphic presentations. However, since pH is in common use, I have not made the transition.

[9] For the relation of free energy to other thermodynamic quantities see table 3.

treatment of energy changes are part of the technicalities of the subject, I shall pursue only the main theme.

It is conceived that the work expended in lifting a stone reappears in the ability of the stone to do work upon falling to its original position. This ability may be on display, or the ability may be latent or potential. Covering both cases is the word energy, defined as the *ability* to do work. When energy is considered without regard for the mechanisms of display it is convenient to characterize a system by its potential energy. Thus arose the concept of potential energy possessed by a given distribution of masses, by a given distribution of electric charges or by chemical substances capable of chemical action.

In the case of a stone upon a shelf the potential energy depends upon the mass and the position. The mass is an extensive property. The potential energy per unit mass is an intensive property. The potential energy per unit mass gives distinction to the particular case. Technically it is called *the potential*.[10] In chemistry the chemical potential of a substance in solution is the potential energy which the solution acquires per chemical equivalent of the substance added; other constituents, temperature and pressure, remaining constant. It can be shown that, if appropriate paths be provided, a mass will fall from a position of higher to one of lower gravitational potential, an electric charge will move from a place of higher to a place of lower electrostatic potential, heat from a body of higher temperature (which is a potential) to a body of lower temperature. If a path be provided a substance will move from a phase where its chemical potential is the higher to a phase where its chemical potential is the lower until at equilibrium the chemical potentials of the substance are the same in both phases.

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[10] Strictly the potential is so defined that a concept of an absolute potential may be had. Since only differences of potential may be measured we may say that the excess of potential at a point A over that at point B with reference to any quantity M is the work per unit of M which must be done in carrying a very small positive amount of M from B to A. (See International Critical Tables.)

The concept of the potential, which was first named by George Green in 1828 and which has found innumerable applications, was introduced to chemistry by Willard Gibbs. His definition of chemical potential is as follows.

"If to any homogeneous mass we suppose an infinitesimal quantity of any substance to be added, the mass remaining homogeneous and its entropy and volume remaining unchanged, the increase of the energy of the mass divided by the quantity of the substance added is the *potential* for that substance in the mass considered. (For the purposes of this definition, any chemical element or combination of elements in given porportions may be considered a substance, whether capable or not of existing by itself as a homogeneous body)."

Gibbs shows that for entropy, volume and energy in the above definition there may be substituted temperature, pressure and the free energy, F. Then $\frac{dF}{dm} = \mu$ or in terms of equivalents, N, $\frac{dF}{dN} = \mu$.

For the purposes to which chemical potentials are put, a chemical potential may be ascribed to a constituent of a compound or even to the escapable electrons within a compound, element, or ion. There will subsist between the chemical potentials of interdependent components of a system in a state of equilibrium the same equation that expresses their stoichiometrical relations. For example, if μ represent a chemical potential and its subscript the species to which it refers, we have

$$\begin{aligned} &H^+ + \epsilon = \tfrac{1}{2}\,H_2 && \text{Stoichiometrical} \\ &\mu_{H^+} + \mu_{\epsilon_1} = \tfrac{1}{2}\,\mu_{H_2} && \text{Chemical potentials} \qquad (1) \end{aligned}$$

$$\begin{aligned} &Fe^{+++} + \epsilon = Fe^{++} && \text{Stoichiometrical} \\ &\mu_{Fe^{+++}} + \mu_{\epsilon_2} = \mu_{Fe^{++}} && \text{Chemical potentials} \qquad (2) \end{aligned}$$

These two oxidation-reduction systems are found in the electric cell depicted in figure 2. They are kept from direct action by an intervening, indifferent solution. When an unattackable metal joins the two solutions it acts as a filter allowing electrons but not other components to escape from the system where the chemical potential of the electrons is the higher to the solution where it is the lower. For the restricted purpose to appear presently there need be no specification of the mechanism by which electrons escape from positions of seclusion within molecules and ions and no consideration of electrostatic relations. Now divide the metal into two parts, called electrodes, and attach these to a potentiometer as indicated. Virtually the readings on the potentiometer have been calibrated in terms of the standard difference of chemical potentials of electrons at terminals of a Weston standard cell which is used in the practical definition of the international volt. When adjustment of the potentiometer is such that equilibrium obtains and no current flows in the studied cell the potentiometer reading is identical with the difference between the chemical potentials of the electrons on the two sides.[11]

If the unit of the potentiometer reading (E) is the volt, which has the dimensions of energy per coulomb, and a chemical potential has the dimensions of energy per chemical equivalent or its electrical equivalent, 96,500 coulombs or one faraday, F

$$FE = \mu_{\epsilon_1} - \mu_{\epsilon_2} \tag{3}$$

It may be shown that when any species[12] is selected from the oxidation-reduction continuum it is possible to use its relation to other species in arriving at the same energy relation that is reached directly by combining equations (1), (2) and (3) namely (4)

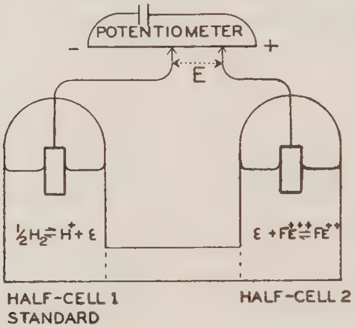
POTENTIOMETER
–
+
E
$\frac{1}{2}H_2 \rightleftharpoons \overset{+}{H} + \epsilon$
$\epsilon + \overset{+++}{FE} \rightleftharpoons \overset{++}{FE}$
HALF-CELL 1
STANDARD
HALF-CELL 2

FIG. 2. ELECTRIC CELL AND POTENTIOMETER

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[11] This use of chemical potentials of electrons is barren of all results other than that desired. For instance, there is no occasion to deal with "single potentials" at isolated electrodes. Guggenheim (J. Phys. Chem. **33**, 29) proposed to differentiate between the chemical potential of a neutral substance and the chemical potential of a charged ion. While this distinction is useful it seems not to be necessary here. The movement of electrons in any one phase is that of a displacement current in a neutral environment or in a uniformly charged system, and where electrical potential jumps occur the energy of transfer across the field is automatically included in the general definition of the chemical potential. When the cell is in electrical (potentiometric) balance there is equality in the chemical potentials of electrons in adjacent phases between which there is no passive resistance to electron transfer. For the situation at liquid junction see footnote 16.

[12] See discussion in Chapter XVIII, *The Determination of Hydrogen Ions*, 3rd edition, Clark, 1928.

$$FE = (\tfrac{1}{2}\mu_{H_2} - \mu_{H^+}) - (\mu_{Fe^{++}} - \mu_{Fe^{+++}}) \qquad (4)$$

FE is the potential energy in volt faradays which would be displayed at constant temperature and pressure were the reaction between the two systems to take place reversibly.

When the configuration of the electric cell is removed the electrode potential is non-existent in actuality but its value remains a convenient expression of that difference between the differences among the chemical potentials of the two systems which determines the possibility of reaction.

To make this energy definite it has to be associated with the participation of definite quantities of the components. The specification usually involves the concentrations of components, but unfortunately there is no universally applicable, simple relation between the chemical potential of a substance and its concentration in a solution. To avoid discussion of the difficulties I ask you to accept a relation[13] derived from the laws of a highly rarefied gas which leads to equation (5) where for simplicity the numerical coefficient for 30° is used.

$$E = E_0 + 0.06 \log \frac{\sqrt{H_2 \text{ pressure}}}{[H^+]} + 0.06 \log \frac{[Fe^{+++}]}{[Fe^{++}]} \qquad (5)$$

Here brackets indicate that the concentration of the indicated species is in molar terms. Fortunately an equation of this type will express most of the relations to be discussed.

A series of comparisons are to be made with the hydrogen system maintained at the standard state of 1 atmosphere hydrogen and 1

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[13] In general for a narrow range of conditions

$$\mu_A = \frac{RT}{n} \ln [A] + B$$

where B is a constant depending on temperature and pressure. [ ] is used to signify concentration of the substance whose symbol is enclosed, concentration being expressed in moles per liter. If μ has the dimensions of energy per equivalent, n is the number of equivalents per mole. With an equation, as above, for each species, and equation (4) there is obtained the following relation where E_0 represents a summary of constants.

$$E = E_0 + \frac{RT}{F} \ln \frac{\sqrt{H_2 \text{ pressure}}\ [Fe^{+++}]}{[H^+] \qquad [Fe^{++}]}$$

normal hydrogen ion concentration (strictly unit activity[14]). Then, since the logarithm of 1/1 is zero the second term on the right of equation (5) drops out. Designating the use of this standard by the subscript in E_h we have

$$E_h = E_0 + 0.06 \log \frac{[Fe^{+++}]}{[Fe^{++}]}$$

or in general for any system involving n equivalents

$$E_h = E_0 + \frac{0.06}{n} \log \frac{[\text{Oxidant}]}{[\text{Reductant}]} \qquad (6)^{15}$$

When the ratio $\frac{[\text{Oxidant}]}{[\text{Reductant}]}$ is unity, $E_h = E_0$, the characteristic constant of the system. Hereafter E_h will be called "electrode potential" or sometimes only "potential."

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[14] The quantity *activity* is substituted for the concentration in the strict equation for the following reason. The equation

$$\mu_A = \frac{RT}{n} \ln [A] + B$$

(See footnote 13) is derived for a solute A behaving as an ideal solute, i.e., one comparable to an ideal gas in which forces of mutual attraction or repulsion between particles play a negligible part in their conduct. Since no solute conforms strictly to this ideal the equation is of very limited applicability. On the other hand the above equation is useful both for restricted purposes and as a limiting equation. Its form may be preserved by employing a correction factor γ_A called the activity coefficient. Then $[A]\gamma_A$ is virtually a corrected concentration called the *activity*. However, the concept of the activity may also be developed in such a way as to involve reference to a standard state. For this see Lewis and Randall (1923).

[15] For simplicity there is given here the equation applicable when one species of oxidant and one species of reductant are present. Presently it will be shown that in the cases of the dye systems a change of pH throws in or out of action one or another undissociated molecule or ion. It then becomes convenient to so formulate the electrode equation that it will contain a term in which are found the concentrations of total oxidant and total reductant and another term containing the acid- or base-dissociation constants and the hydrogen ion activity. See example in footnote 17. For a discussion of such, more complete equations see Clark, *The Determination of Hydrogen Ions*, 3rd edition, Chapter XVIII, Baltimore, 1928, or Studies on Oxidation-Reduction II by Clark and Cohen, Public Health Reports, **38**, 666, 1923. Equation (6) without restriction as to temperature is:

$$E_h = E_o + \frac{RT}{n\,F} \ln \frac{[\text{Oxidant}]}{[\text{Reductant}]}$$

where R is the gas constant, T the absolute temperature, F the faraday and *ln* signifies logarithm to the base e.

I have dodged consideration of phenomena at liquid junctions[16] and shall assume that when there is used an intervening saturated solution of potassium chloride there remains an indefinable effect upon E which is believed to be sufficiently constant to be included in the practical definition of the standard of reference.

When two systems are mixed a catalyst may be required to put their reaction into display. Even the standard system requires a catalyst, platinum black, to activate the process $1/2\ H_2 \rightleftharpoons H^+ + \epsilon$. In the discussion of those experimental data which immediately follow it will be assumed that there is no passive resistance; that reactions are unrestrained and reversible. Graphic presentations of the relation between electrode potential and *concentrations* of components, rather than chemical potentials of components, will be used to illustrate how electrode potential differences determine the course of an unrestrained reaction.

In figure 3 the ordinate is electrode potential, E_h; the abscissa is per cent reduction. Centers of circles represent experimental points. The curve for the thallium system is flatter than the curve for the iron system because two equivalents ($n = 2$) are involved in the first instance and only one in the second. All such curves are asymptotic to the potential axis at 0 per cent and 100 per cent reduction. Since the electrode potential is then indefinite there is no meaning to the electrode potential of a pure oxidant or pure reductant. We deal only with electrode potentials of *systems*; although, for convenience, a system may be given the name of one component. Since two or more systems at equilibrium in the same medium can have but one electrode potential, each level of potential on this chart defines the per cent oxidation of each of the two systems when mixed. The curves show that thallous ions and ferric ions cannot react appreciably but that thallic ions and ferrous ions can. On addition of a thallic salt to a solution of a ferrous salt the potential will remain on the lower curve until all ferrous ions are oxidized. Then the electrode potential will jump to the upper curve. The potential jump is the end-point of an analytical titration. Of more interest than the analytical end-point is the general use of such curves to show: first, the direction in which two systems can interact; second, the extent of interaction. For the latter purpose it is also necessary to know the amounts of the components. The

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[16] The absence of an equilibrium at the liquid junction precludes a rigid treatment of any cell. However, attainment of infinite delay in admixture of two oxidation-reduction systems can be approximated, as when the process of action is segregated at the electrode. The troublesome effect of unequal migrations of oppositely charged ions at a liquid junction can be practically eliminated by such a choice of conditions as will make the two liquids in contact practically the same or it can be reduced by judicious use of saturated KCl-solution, etc. One of the most important functions of the intervention of materials at liquid junctions is to prevent that direct action of two oxidation-reduction systems which would lead to equivalence of chemical potentials of electrons on the two sides of the cell.

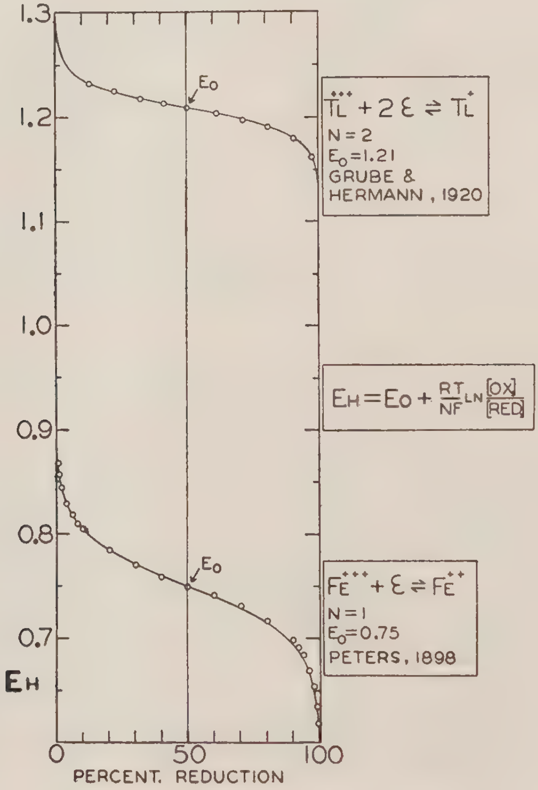

Fig. 3. Relation of Observed Electrode Potential E_h to Per Cent Reduction in the Cases of Two Inorganic Systems

amounts determine what is called the capacity factor. A difference of potential is called the intensity factor.

There have been many such comparisons of inorganic systems but little attention was given to organic systems prior to 1920. In that year occurred a remarkable coincidence. Practically simultaneously Granger (1920) published a dissertation from Nelson's laboratory at Columbia University, Clark (1920) reported on two dyes and Biilmann (1920) described his first work with "the quinhydrone electrode." At essentially the same time Conant was formulating a project on which he first reported in 1921 (see Conant, Kahn, Fieser and Kurtz, 1922) and he has told me evidence that another investigator was then in the field. Thus at least four laboratories and doubtless more began essentially at the same time independent investigations of the electrode potentials of organic systems. Of Gillespie's part I shall speak later.

EXAMPLES OF CONFORMITY OF EXPERIMENTAL DATA TO THE TYPE CURVE (N=2)

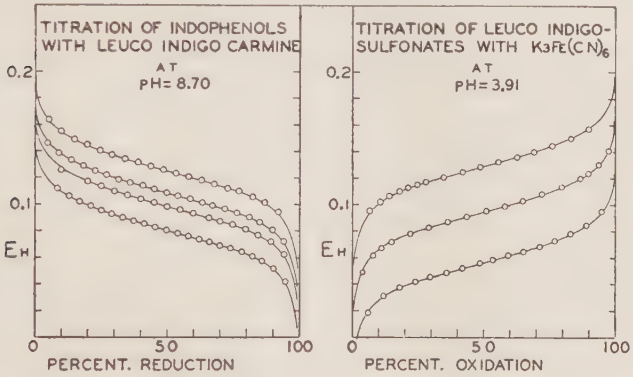

FIG. 4

I shall have to select from a large body of data; I shall have to be brief, and I hope to be pardoned for using material from my own laboratory and for failing to mention many prominent contributors and contributions to the general subject.

In figure 4 are charted a few experimental data and the corresponding curves of theoretical form. Please accept these as fair examples of the conformity of experimental data with a theoretical relation so that I shall not have to confuse the main outlines of the subject by showing the innumerable experimental details.

In figure 5 are assembled the curves for several dye systems at pH 7. There are included one system studied by Conant and Lutz (1924), two studied by Michaelis and Eagle (1930) and 22 of the 64 systems measured in my laboratory (see Studies on Oxidation-Reduction). We have here a set of indicators comparable with acid-base indicators but applicable to a very different category of reactions. A dilute solution of the reductant of any one of these dyes is usually nearly colorless and the degree to which the system is reduced can be measured colorimetrically. This degree and the characteristic potential at known pH permit a colorimetric estimate of the electrode potential of a system with which the dye system is in equilibrium. Later there will be mentioned the uses of these systems in exploring the living cell and as electromotively active systems which act as mediators in transferring to the electrode the electrode potential function of the chemical potentials of electromotively inactive systems.

In placing the curves of figure 5 it was necessary to specify a particular level of pH because of a complication which permeates the entire subject. This may be illustrated by the data for the methylene blue system outlined in figure 6. At B are curves relating potential to per cent oxidation; in each case at constant pH. When the potentials at the centres of these curves are plotted against pH there is obtained curve A. Obviously we have to do with three dimensions: potential, per cent oxidation and pH. To coördinate these place diagrams A and B perpendicular to one another as shown in C and move the sigmoid curves to their proper positions on the other curve. There is then outlined the surface in three dimensions indicated in D.[17] This will aid in visualizing the course of systematic experiments. First, buffer the solution at constant pH and measure the change of the electrode potential with change in the per cent oxidation. Second, repeat the procedure at each of several levels of pH or else carry a fixed mixture of oxidant and reductant through various levels of pH.

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[17] The equation for this surface is:

$$E_h = E_o + 0.03 \log \frac{[S_o]}{[S_r]} + 0.03 \log (K_{r1} K_{r2}[H^+] + K_{r2}[H^+]^2 + [H^+]^3)$$

Where $[S_o]$ = concentration of total methylene blue
$[S_r]$ = concentration of total methylene white
K_{r1} and K_{r2} are acid dissociation constants of cations $H_3{}^+NRNH_3{}^+$ and $H_2NRNH_3{}^+$ of methylene white.

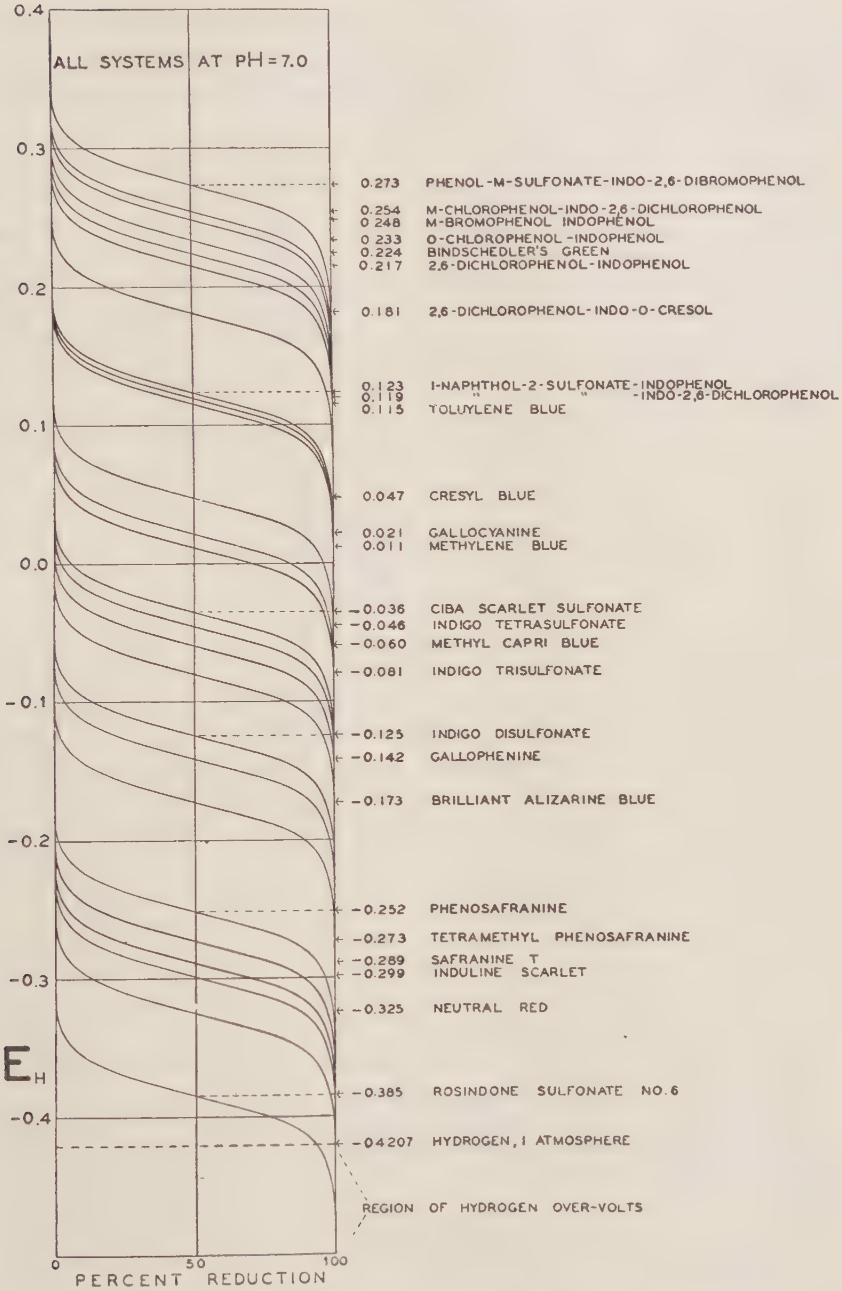

FIG. 5. OXIDATION-REDUCTION INDICATORS AT pH 7.0

Since the curves relating potential to per cent oxidation at constant pH are usually uniform, conform to type and vary only with the number (n) of equivalents concerned, it usually[18] suffices to give the

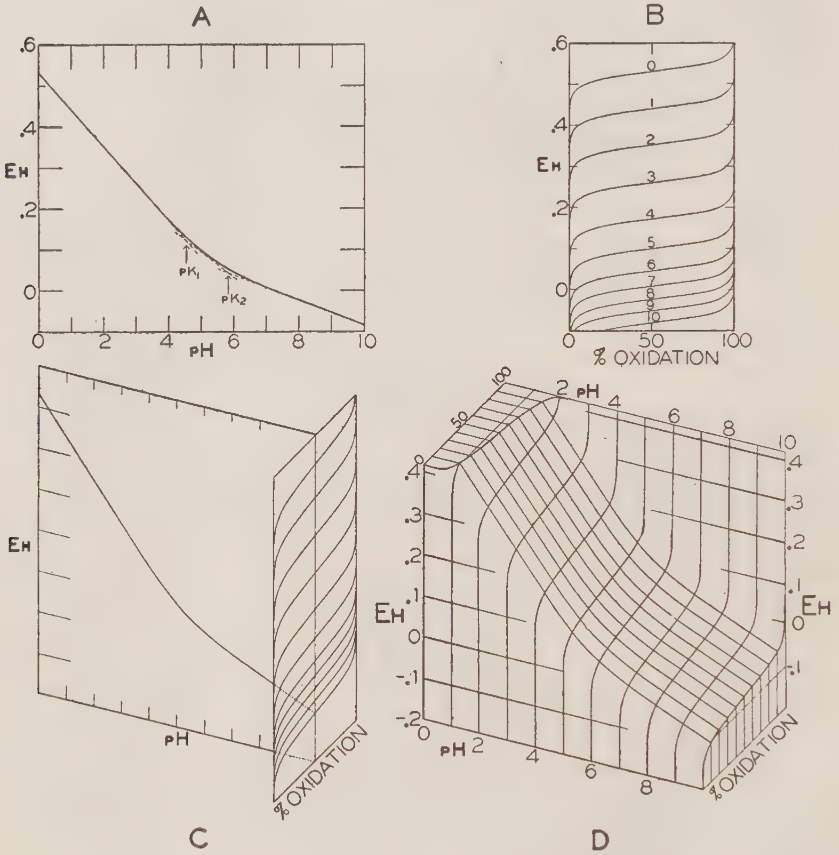

Fig. 6. The Methylene Blue-Methylene White System

A. Relation of electrode potential at 50 per cent oxidation to pH.

B. Relation of electrode potential to per cent oxidation at each of several levels of constant pH (unit intervals of pH).

C. Figures A and B mutually perpendicular in isometric projection.

D. Isometric projection of the surface on which E_h, pH and per cent oxidation are related. Also surfaces cut by a plane of constant pH (at pH = 10) and by a plane of constant potential (at E_h = 0.42) relation between pH and the potential at 50 per cent oxidation. Such relations are shown in figure 7. Since similar curves will be shown later, please remember that here the electrode potentials apply to each system at 50 per cent oxidation. The particular position of a change in the slope of any one of these curves is due to the distinctive acid base dissociation constants of the components concerned.

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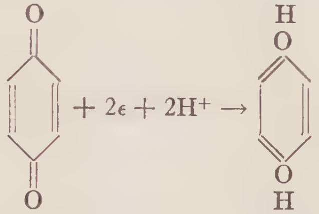
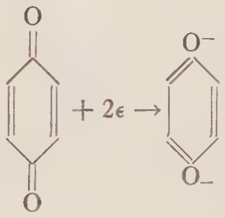
[18] Several distinct sorts of qualifications have been observed.

1. Formation of complexes with other constituents of the solution. See for example Peters, 1898.
2. Formation of aggregates. See Stiehler and Clark, 1933.

The explanation of the main pH effect is simple in principle and may be made clear if we resort to a mechanistic conception of what takes place in a half-cell containing quinone, joined to the standard half-cell. Electrons come from the standard system *via* the metallic connection; but hydrogen ions are also needed to form hydroquinone if the reaction is:



In very acid solution hydrogen ions are abundant and their acquirement involves comparatively little energy. As pH increases more energy is used in withdrawing hydrogen ions from the acid-base continuum and hence less energy is set free in the reduction of quinone by the standard system. Finally, at very high values of pH it is as if the process became simply:



See figure 8.

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3. Formation of quinhydrones, meriquinones or semiquinones. See discussion by Michaelis (1931–1932) and reference to previous treatments of meriquinones.
4. Environmental effects, "salt-effect," etc.

See *Studies on Oxidation-Reduction.*

For many of the biochemical uses of oxidation-reduction indicators the details of their conduct with respect to one or another of the above effects and in particular with respect to combinations with proteins are not yet adequately known.

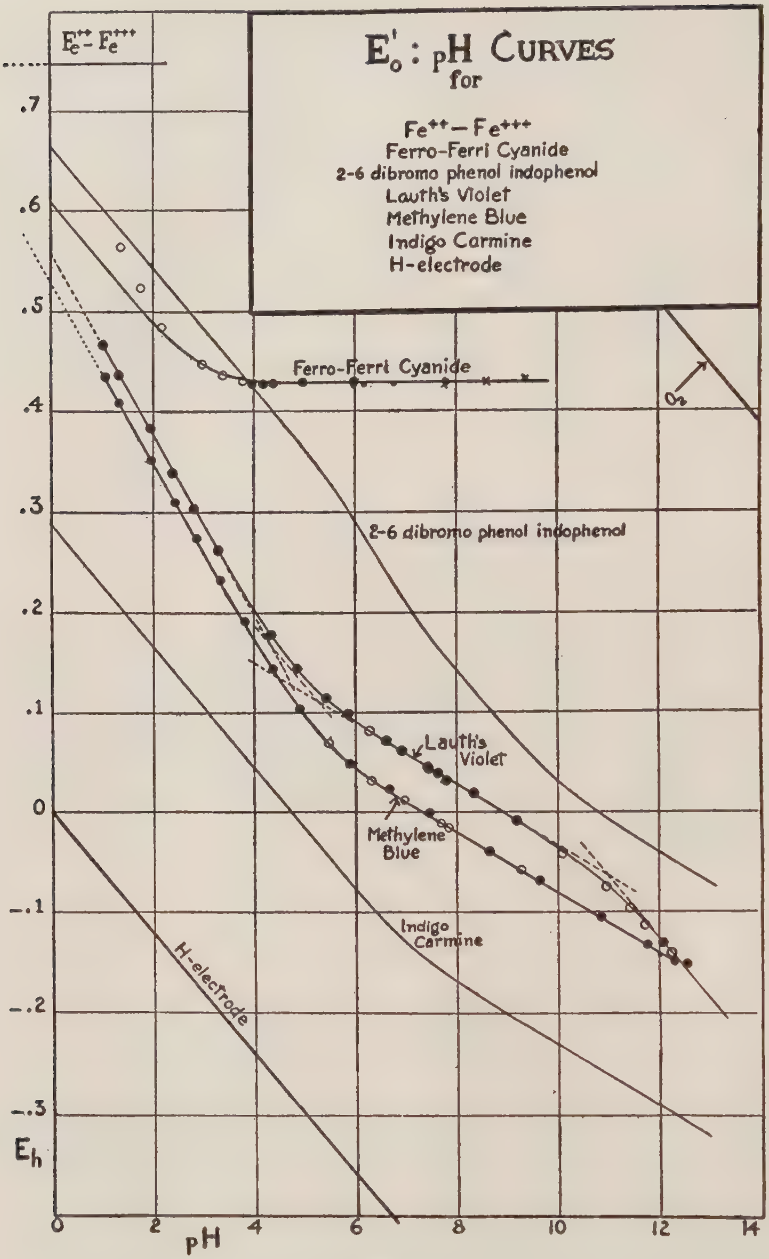

E_0' : pH CURVES
for
$Fe^{++}-Fe^{+++}$
Ferro-Ferri Cyanide
2-6 dibromo phenol indophenol
Lauth's Violet
Methylene Blue
Indigo Carmine
H-electrode
$Fe^{++}-Fe^{+++}$
Ferro-Ferri Cyanide
2-6 dibromo phenol indophenol
Lauth's Violet
Methylene Blue
Indigo Carmine
H-electrode
O_2
.7
.6
.5
.4
.3
.2
.1
0
-.1
-.2
-.3
E_h
0
2
4
6
8
10
12
14
pH

FIG. 7

Both the energy of "dilution" of hydrogen ions and the energy of ionization of the components of the oxidation-reduction system have to be considered.

In the pH effect are several matters of direct and theoretical interest to biochemists. I shall mention only three.

In dealing with the energy changes of metabolic processes it would not do, for instance, to consider only the potential free energy of lactic acid since this substance appears in tissues as lactate ion. The energy change of ionization must be considered. Also, if the secondary alcohol of lactate is oxidized to the ketone of pyruvate there will enter a pH effect comparable to that of the quinone system in acid solution.

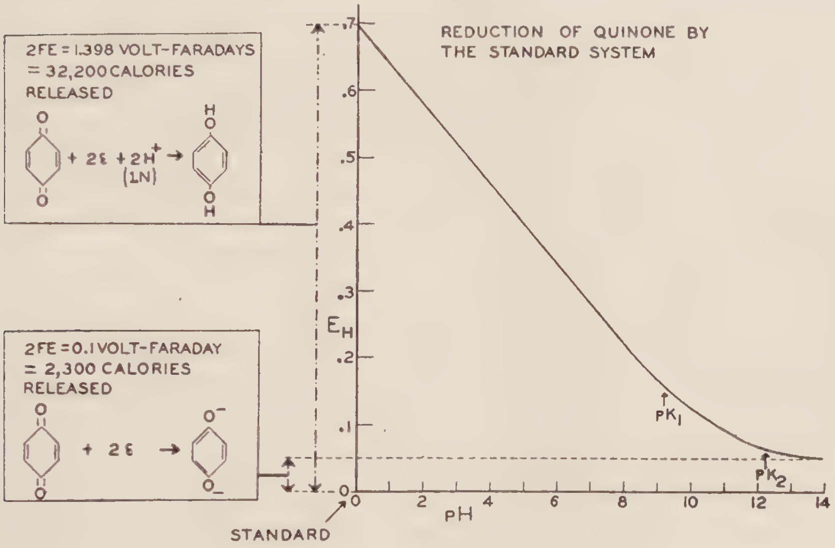


FIG. 8

Another aspect may be illustrated by means of the data for an indophenol system. See table 1.

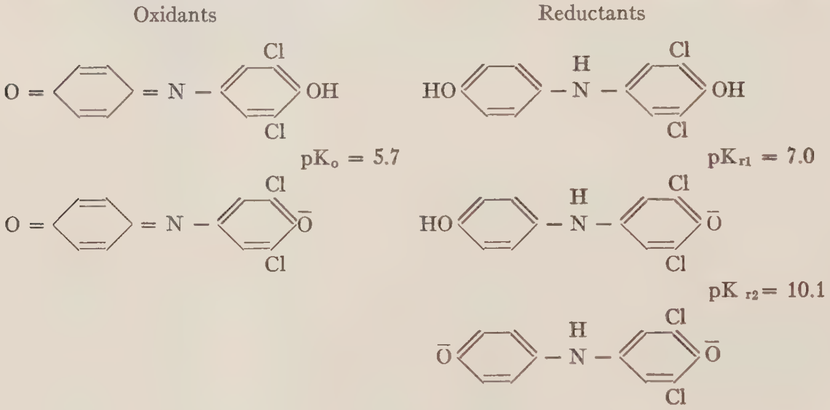
The five species shown make six interlocking systems the resultant potentials of which may be described with the one equation:

$$E_h = E_o + \frac{RT}{2F} \, ln \, \frac{[S_o]}{[S_r]} + \frac{RT}{2F} \, ln \, \frac{K_{r1}K_{r2}(H^+) + K_{r1}(H^+)^2 + (H^+)^3}{K_o + (H^+)}$$

Because of changes in the ionization constants which attend reduction, the following relation obtains. (See table 1.) When one mole of the dye is reduced in a buffer of pH 6.5, there is released to the buffer system 0.62 mole of base. Now the special sort of oxidation-reduction process here concerned is very different from that involved in the addition of oxygen to and removal of oxygen from the blood pigment. Yet in each case there are integrated acid-base equilibria and oxidation-reduction equilibria. In the blood, removal of one mole of oxygen from oxyhemoglobin is accompanied by release of about 0.6 mole of base. The double function of the blood pigment as a carrier of oxygen to the tissues and as a virtual carrier of base to meet incoming bicarbonate ions is a beautiful biological adaptation of a chemistry which is still somewhat obscure. In the case of the indophenol we have a clarify-

TABLE 1

Case of 2,6-dichlorophenol indophenol



At pH 6.5 1 mole oxidant	"binds"	0.86 mole B^+
At pH 6.5 1 mole reductant	"binds"	0.24 mole B^+
Made available by reduction		0.62 mole B^+

Case of Blood Pigment

At pH 7.3 1 mole oxyhemoglobin	"binds"	1.87 moles B^+
At pH 7.3 1 mole hemoglobin	"binds"	1.23 moles B^+
Made available by reduction		0.64 moles B^+

ing, if incomplete, analogy, which may or may not hold when the chemistry of the blood pigment is made better known.

As already explained the description of a system by an equation or diagram requires of the experimental work systematic control of the individual factors. Now imagine a natural process in which there is no such variation of one factor at a time but rather what would correspond to a progression on the surface of figure 6 D without restraint to a particular coördinate. It would be then as if the distinctions of the systematic experiments were lost. Indeed it can be made to *appear* as if a change in pH were accomplishing the oxidative process.

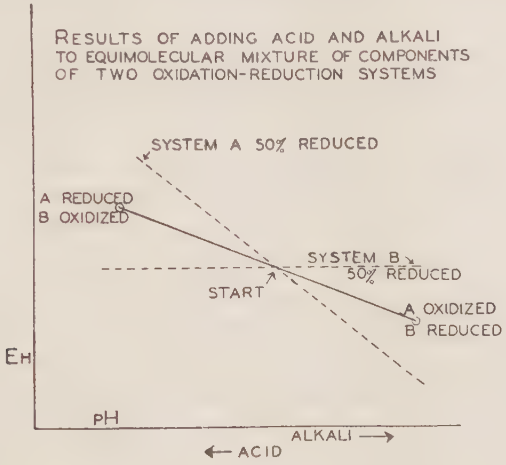

RESULTS OF ADDING ACID AND ALKALI
TO EQUIMOLECULAR MIXTURE OF COMPONENTS
OF TWO OXIDATION-REDUCTION SYSTEMS
SYSTEM A 50% REDUCED
A REDUCED
B OXIDIZED
SYSTEM B
50% REDUCED
START
A OXIDIZED
B REDUCED
EH
pH
ALKALI ⟶
⟵ ACID

FIG. 9

From data now available several specific instances of the type shown in figure 9 may be used in striking experiments.[19] Here are indicated two oxidation-reduction systems whose characteristic potential: pH curves cross. Addition of acid brings about the oxidation of system B and the reduction of system A; while addition of alkali reverses the process. This is only an example of that integration between the equilibrium states of the two categories which may someday serve as a model in the analysis of distinct changes in oxidative metabolism which accompany great changes in the acidity of a bacterial culture or to reveal advantage in the constant pH of our own tissues.

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[19] Lecture experiments may be performed with: (1) A mixture of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, the latter in excess, and 2,6-dichlorophenol indophenol. This dye is also an acid-base indicator, red in acid solution, blue in neutral and alkaline solutions. (2) Alloxantin and methylene blue.

At this point I should like to interject a topic somewhat apart from the theme of this discussion. In addition to these interrelations between acid-base *equilibria* and oxidation-reduction *equilibria* there are well known relations between acid-base *equilibria* and the kinetics of hydrolysis by enzymes and acids. Recently interconnection has been extended by a flood of notes upon the modification of rates of certain hydrolytic processes by oxidation or reduction of "activators" of the enzymes concerned. Waldschmidt-Leitz calls the oxidation and reduction of the activator glutathione the natural control of these special, hydrolytic enzymes. However, Hellerman, Perkins and Clark (1933) have demonstrated that oxidizing agents and certain specific agents modify the action of urease reversibly (not in the thermodynamic sense of "reversibly"). The agents attack, not an extraneous "activator," but Sumner's crystalline urease[20] itself. A specific chemistry not yet brought within the general treatment is involved. Bersin and Logemann (1933) have shown a similar effect in the case of the proteolytic enzyme papain. This we have confirmed. See table 2. Thus a cycle of interconnection, still very limited, is found for certain aspects of three great classes of processes; acid-base exchange, oxidation-reduction and hydrolysis. If the interconnections can be consolidated and carried into the practical study of the living cell there will be met the demands of a biochemistry which is true to its vision of life's coördinations.

Before considering the extension of electrode potential measurements to the characterization of definite systems of natural occurrence, I should like to prepare the way to a subsequent discussion by reviewing measurements of cell suspensions and cell interiors. During this review please keep in mind the fact that they are actual physical measurements which are mentioned and not their usual chemical translation.

In 1915 Gillespie (see Gillespie, 1920) found that saline and broth suspensions of bacteria change the electrode potential progressively with time as illustrated in figure 10. With Dr. Gillespie's permission I extended his work in 1919. The results were rich in suggestions regarding the influence of various species, various media and various conditions of growth; but the impressive difficulties were these. Although time: potential curves could be reproduced roughly, the potentials were rather precarious. Those of aerated cultures behaved suspiciously like the potentials of the notoriously ill-behaved, oxygen electrode in an indifferent medium. Adequate chemical interpretations of the physical data could not be made. The data were shelved to await confirmation. Thus originated our study of the indicator method; a field of inquiry which Paul Ehrlich had entered in 1885 before theory had ripened the time. In the following six years Clark and Cohen (1920–1925) and Cannan, Cohen and Clark (1926) and subsequently many others, using alternately and in conjunction the direct electrode method and, as it became developed, the indirect indicator method, established confidence in the following conclusions. While suspensions or cultures of bacteria, yeasts and various other

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[20] Sumner's crystalline preparation is either urease itself or an association between the enzyme and a protein which is so close that separation in a strict chemical sense has not yet been accomplished.

TABLE 2

Reversible inactivation of hydrolytic enzymes

UREASE		PAPAIN	
Conditions	Relative activity	Conditions	Relative activity
Control	100	Control	100
$O_2 + Cu^{++}$	40	$O_2 + Cu^{++}$	55
Above; after:		Above; after:	
Thioglycollate	100	Glutathione	100
Glutathione	100	H_2S	100
Control	100	Control	100
+ Iodine	70	+ Iodine	40
Above; after H_2S	100	Above; after:	
		H_2S	100
		Glutathione	100
Control	100	Control	100
+ Cu_2O	0	+ Cu_2O	0
Above; after H_2S	100	Above; after H_2S	100

Hellerman, Perkins and Clark, 1933

cells produce but little material which we may call electromotively active, they exhibit an ability to repeatedly mobilize such material as if from a large reserve of metabolites. In the absence of oxygen the electrode potential may approach or even fall below that of the hydrogen electrode. In the presence of oxygen the electrode potential fluctuates in a zone surprising with respect to its position. I shall discuss this presently.

The next step was the exploration of the cell interior. The first explorers were Joseph and Dorothy Needham (1925). They were followed by Rapkine and Wurmser (1925–27) and others. Especially able teams were led by Dr. Cohen, who is familiar with the properties of the indicators, and Dr. Chambers, a cytologist who is a master of the micro-injection technique. Their main conclusions are as follows. When *Amoeba dubia*, for instance, is in nitrogen the cell interior reduces instantly the indophenols of more positive characteristic potentials and *usually* reduces less and less rapidly the indicators of progressively lower characteristic potentials. Dr. Cohen and Dr. Chen (1933) have recently found evidence for a limiting level indicated in figure 11.

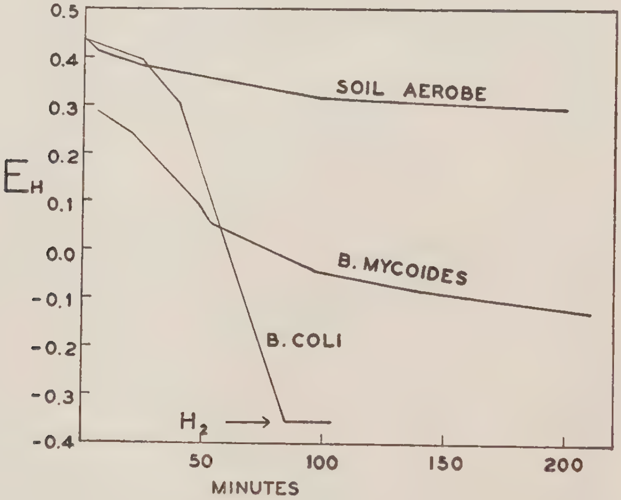

Fig. 10. Saline and Broth Suspensions of Bacteria. Variation of Potential with Time
After Gillespie, 1920

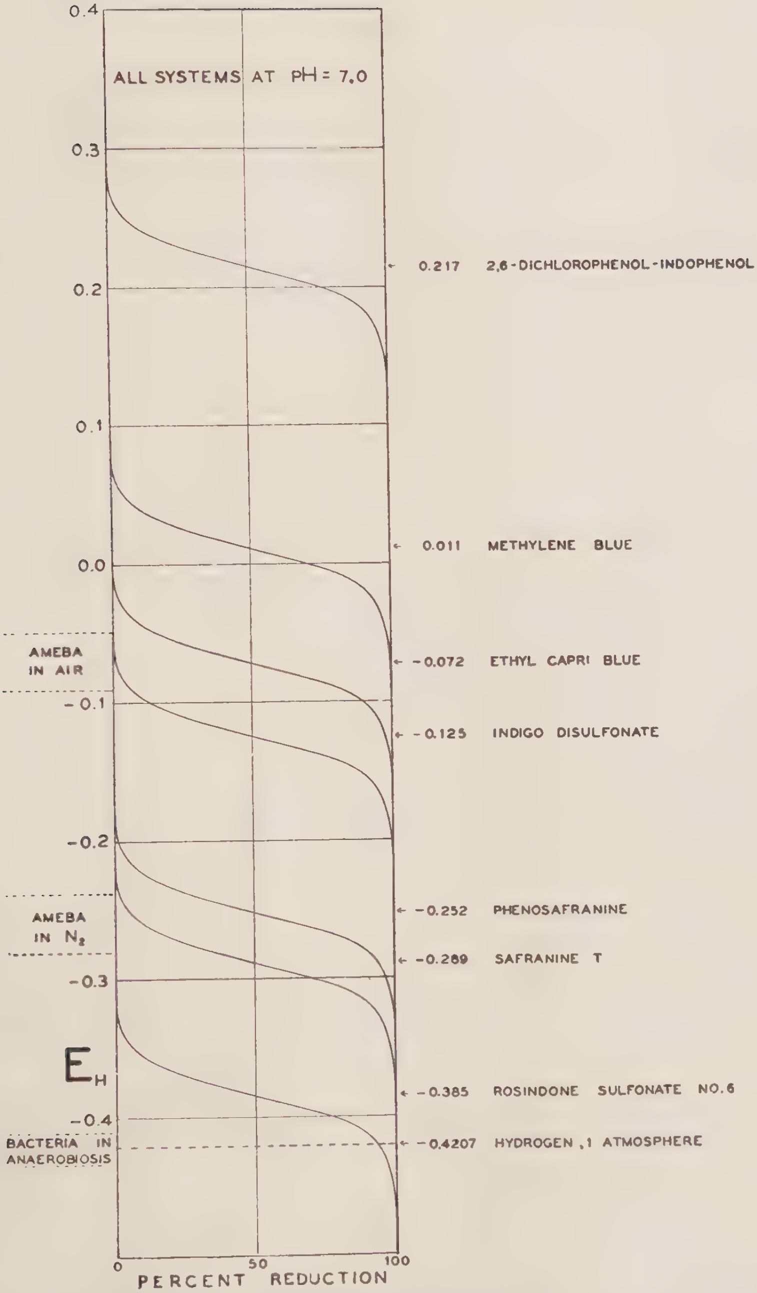

FIG. 11. OXIDATION-REDUCTION INDICATORS AT pH 7.0 AND THEIR APPROXIMATE INDICATIONS OF THE ZONES OF POTENTIAL OBTAINING IN THE CELL OF AMOEBA DUBIA. ALSO ZONE OF POTENTIAL INDICATED BY ELECTRODE MEASUREMENTS OF CERTAIN ANAEROBIC BACTERIAL CULTURES

Needless to say the conclusions have been guarded by consideration of the specific properties of particular dyes and numerous other considerations. This case represents the general trend of all cells in anaerobiosis and only the general trend will be considered now.

Much more remarkable is the conduct of the cell in an aerated culture. The "potential" of *Amoeba dubia* fluctuates in a region (indicated in figure 11) far distant from what it should be were there even remote approach to equilibrium with appreciable pressures of oxygen. This case illustrates a general tendency exhibited by other cells and by cell suspensions in general.

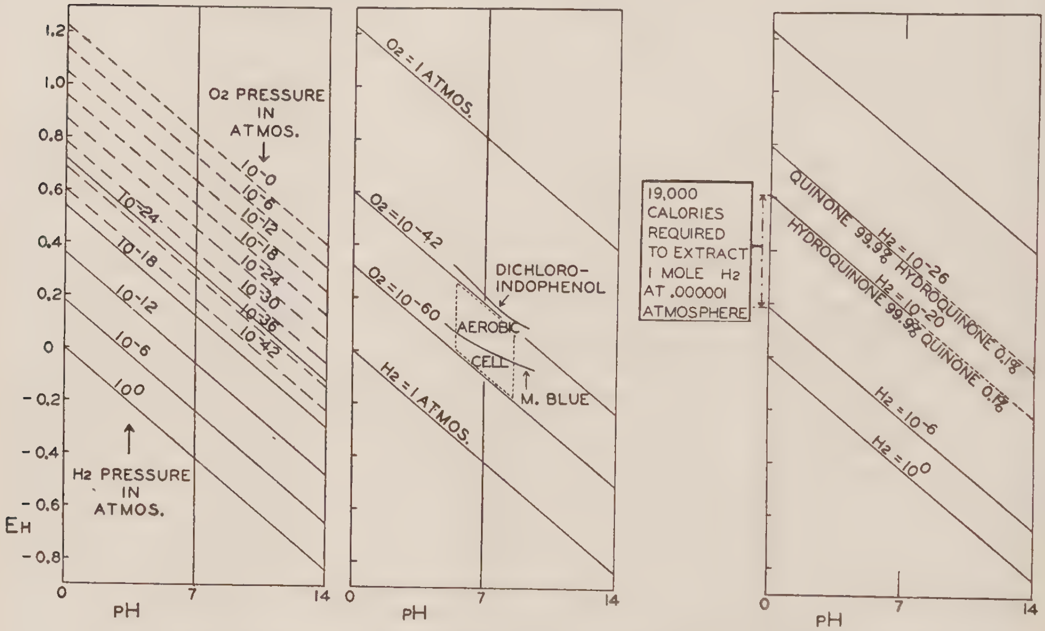

Fig. 12. Theoretical Equilibrium Relations between E_h, pH, Oxygen Pressure and Hydrogen Pressure; Together with Placements of Several Oxidation-reduction Systems

This situation demands brief theoretical treatment. In figure 12 the lowest diagonal represents the potential of the hydrogen system as a function of pH, the hydrogen pressure being maintained at one atmosphere. Neglecting the practical difficulties of the experimental demonstration we dare assert on theoretical grounds that when the hydrogen pressure is lowered to one millionth of an atmosphere the potentials should rise to the next highest diagonal. Each successive diagonal represents the lowering of the hydrogen pressure six decimal places. Likewise the uppermost diagonal represents the oxygen system ($O_2 + 4H^+ \rightleftharpoons 2\ H_2O$) with oxygen at one atmosphere and each shift downward represents a shift of 6 decimal places in the pressure of oxygen. When the potential is in the zone attributed to the aerated cell the calculated oxygen pressure should be of the order of 1×10^{-42} to 1×10^{-60} atmosphere, *were there a true equilibrium.* While this "calculation value" obviously has no significance of a physically real pressure it expresses quantitatively the well recognized fact that cells are remote from equilibrium with oxygen at air pressure.

It also defines a problem in catalysis; namely, how is it that oxygen can be activated without putting into play that full capability which would destroy the cell?

It also defines a problem in the practice of anaerobic culture. Since cells can maintain certain dyes in their reduced states even when pure oxygen is bubbled through the suspension, what becomes of the indicator test for the anaerobic state? It becomes a problem of establishing reasonable assurance of a true equilibrium between the dye system and that vanishing quantity of oxygen which means practically the absence of oxygen. While there is no point in assigning one of the calculation values, we have reasonable assurance that approaches to equilibrium have occurred and that Pasteur was essentially right when he remarked that in certain instances the "last atom" of oxygen has been removed from an anaerobic culture.

While figure 12 is before us let us consider an experiment by Wieland which is often quoted as showing a model of a "hydrogen transporting enzyme." Wieland claimed that he had demonstrated the removal of hydrogen from hydroquinone by palladium. However, it may be shown with the aid of figure 12 that so large an amount of energy would have to be expended in removing hydrogen, as molecular hydrogen, from hydroquinone that the mechanism originally postulated is highly improbable. Gillespie and Liu (1931) not only have discussed this but they have repeated the experiment and have given another interpretation to those confirmed results which quite naturally led Wieland to his conclusions. It would be unfair to Wieland's theory and to the subject to leave this one experiment in isolated prominence with the above comment. What the case illustrates is the principle that, when the characteristics of a particular system in a given oxidation-reduction continuum are known, it is possible to give a judgment of the probability that this particular system is operating. If, in several cases, the ground be shifted to the postulate that hydrogen, in unspecified form, is transferred in a coupled reaction with coupled energies, at least one source of misunderstanding is eliminated.

To return to the discussion of cell suspensions: In the expression "the electrode potential of a cell suspension" an ambiguity arises only when there is confusion between the physical measurement and its usual chemical interpretation as a function of the chemical potentials of a definite system. I have been discussing only actual physical measurements. If these actualities are fortuitous they deserve no further consideration. Since they have not yet been associated definitely with known equilibrium states of fixed or transient systems they cannot be given the ordinary interpretation. But to the extent that these actual physical measurements may be related to definite biological events they command respect as having met *biological* criteria of significance. Let me illustrate what I mean by "biological criteria."

It is well known that in the chlorination of water or iodation of a wound, the halogen attacks both the living organisms and the dead organic matter. Some years ago we showed (see Clark, Cohen and Cannan, 1925) that the electrode potential of any one chlorinated *or* iodated medium could be used in a very rough empirical way as an indication of residual halogen with which disinfection rates could be correlated. Now it is conceivable that if iodine be added at a rate equal to its reduction or absorption by the constituents of the medium and the products of metabolism, the bacteria present may escape destruction. To this end we introduced an automatic potentiometer which, through relay and pump, controlled the addition of iodine,—never allowing the electrode potential to rise to the region distinctly positive to that of the aerobic cell. While the chemical interpretation of the events was too vague to admit the publication of this experiment, I think it admissable to say that the record of the automatic potentiometer had this significance: its line of fairly constant potential represented a balance of opposing tendencies in which the bacteria survived and apparently thrived in spite of the total addition of many times that amount of iodine, which, if added initially, would have sterilized the culture.

Schmelkes (1933) has made a much more thorough investigation of disinfection by various chloro compounds and has correlated in a very interesting way their chemical properties, disinfectant action and the empirical potential measurements taken during tests.

Or consider the assumption that anaerobic bacteria require an environment having a physically measurable electrode potential distinctly lower than that suitable for an aerobic organism. Several investigators have reported correlations of this sort and Fildes (1929) has gone so far as to relate sporulation of and therefore infection by the tetanus bacillus with the potential of the site of infection.

Numerous other correlations[21] between indicator or electrode measurements and biological events have opened chemical problems which may be long in the solution. In attempting chemical interpretations it may be well to remember that without the definite materials furnished by the fundamental art and science of chemical isolation the biochemist is left in a position worse than that which led some wag to say that a physical chemist is one who makes painfully exact physical measurements upon frightfully impure chemical compounds. Therefore the more significant advances in our subject will be found on turning to measurements of isolated oxidation-reduction systems of natural occurrence. Most of the available data are summarized in figure 13.

Measurements with epinephrine and related compounds required a special mode of operation because the oxidants are very unstable. See Ball and Clark (1931) and Ball and Chen (1933). A buffered solution of epinephrine, for instance, and a solution of an oxidizing agent are run from calibrated bulbs to a mixing chamber. The mixture then passes a series of electrodes and a junction with a reference half-cell. Since the oxidant progressively decomposes, the mixture is changing in composition; yet with a constant rate of flow the composition at any one electrode remains the same and so does the potential at that electrode. The change of potential from electrode to electrode is that which is

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[21] The literature on this aspect of the subject is already so extensive that its critical review would require considerable space. Here I attempt only to indicate both the legitimate nature of this type of investigation and the difficulties of chemical interpretations.

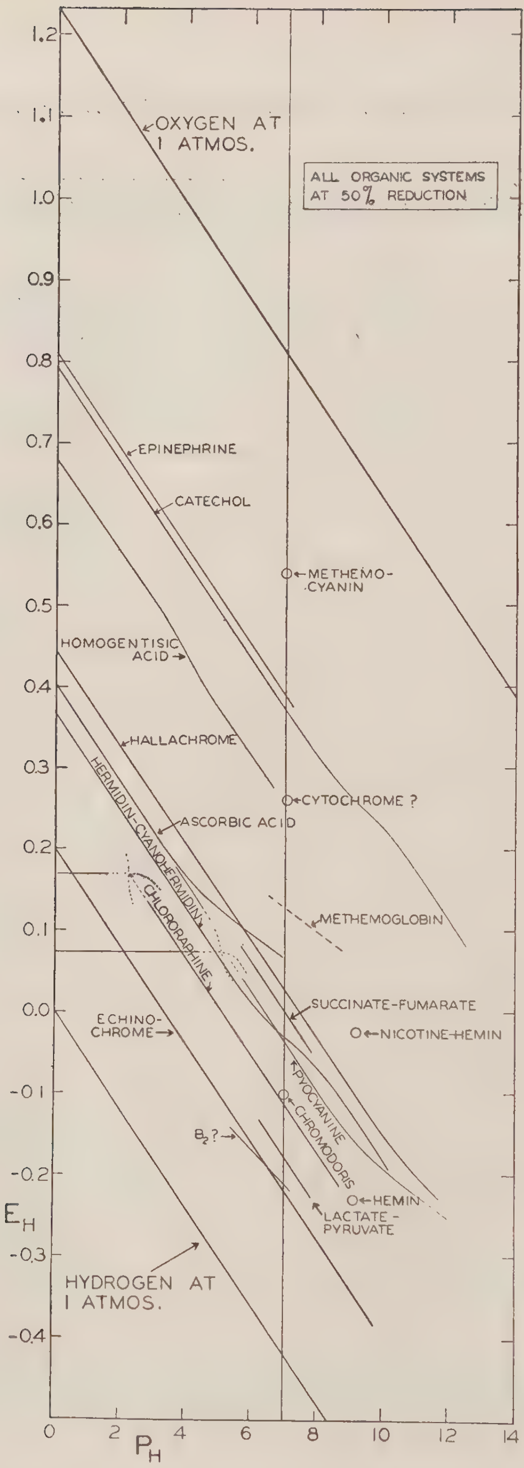

FIG. 13. POTENTIALS OF SYSTEMS OF NATURAL OCCURRENCE, AT 50 PER CENT REDUCTION

to be expected were the oxidant decomposing in accordance with a first order reaction. From this and the rate of flow there can be calculated a good approximation of the potential of the initial mixture. The results satisfy the ordinary criteria of significance. Also the rate of decomposition may be estimated. In neutral solution the oxidant of epinephrine has a half-life of about 0.06 second.[22] Therefore if this hormone is to be protected from an oxidation which would mean immediate destruction, the native environments in which it is found must have potentials distinctly negative to the characteristic of this system shown in figure 13. This and similar arguments confirm the conclusion already reached by the direct measurements.

Included in figure 13 are preliminary measurements of ascorbic acid, vitamin C (Borsook and Keighley 1933) and a substance [said to be related to vitamin B_2 (Bierich, Lang, and Rosenbohm, 1933)] which is one of Warburg's catalysts (Warburg and Christian, 1932).

Cannan (1926–7) opened the investigation of natural pigments other than hemochromogens with his study of hermidin and echinochrome. Since then several other natural pigments have been found to be components of reversible oxidation-reduction systems. [See, for instance, Priesler (1930) and Friedheim (1933).] Friedheim and Michaelis (1931) and Elema (1931) have independently found that pyocyanine, a pigment produced by *Bacillus pyocyaneous*, is reduced in acid solution in two stages involving one equivalent each. They have concluded that the intermediate compound is a free radical. Elema (1933) finds a similar case in the related pigment chlororaphine and Michaelis (1931–33) finds other cases among synthetic systems including some of the systems thought to form meriquinones. Separation to different energy levels of equivalents which usually go in pairs evidently has a mechanistic image and this may have consequences which Michaelis (1933) and Shaffer (1933) hope to develop.

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[22] Ball and Chen (1933) have emphasized with such cases the origin of certain artifacts. For instance, the ordinary use of the characteristic potentials of the epinephrine and dichloro indophenol systems would lead one to say that epinephrine cannot appreciably reduce the indophenol. As a matter of fact it does do so slowly. While complications enter, the origin of this phenomenon lies in the fact that, when the indophenol produces the predicted, very small degree of oxidation, the oxidant of epinephrine decomposes and thus leaves the potential of the mixture at a relatively low level where the indophenol continues to cause oxidation.

While great technical difficulties have prevented accurate measurements of the hemoglobin-methemoglobin system, the data of Conant and his students (Conant, 1923; Conant and Scott, 1926; Conant and Pappenheimer, 1932) have given a clear picture of methemoglobin as the blood pigment with its iron in the ferric state and they have provided a rational basis for the explanation of several processes in which the formation of methemoglobin is concerned. Sometimes there are involved very complicated sets of shifting equilibria.

Of equal interest are a few results obtained with other hemochromogens and hemins. Potential measurements of these were initiated by Conant, Alles and Tongberg (1928). In this field the fundamental researches of Hans Fischer on the porphyrins and hemins and the fundamental researches of Otto Warburg on cellular respiration are converging toward a definite identification of the respiratory enzymes. Special hemochromogens are fast becoming associated with oxidase, peroxidase and catalase functions and also with electromotive activity which reveals them as components of reversible oxidation-reduction systems.

The following results are perhaps the most significant in the whole field. A mixture of succinate and fumarate by itself will not act readily upon a reversible system such as methylene blue. However, Quastel and Whetham (1924) using an enzyme of *Bacillus coli*, and Thunberg (1925), using an enzyme from muscle, found that these enzymes catalyze the establishment of an equilibrium between the succinate-fumarate system and the methylene blue-methylene white system. When the equilibrium constant is combined with the characteristic potentials of the methylene blue system it is possible to calculate the free energy of oxidation of succinate to fumarate. See table 3. The system even in the presence of the activating enzyme alone gives no satisfactory electrode potentials. However, the methylene blue, thionine or indigo tetrasulfonate system can act as a mediator and the potentials then satisfy criteria of significance. By this means Lehmann (1930) and later Borsook and Schott (1931) measured the characteristic potentials and hence the free energy change. Borsook and Schott also used thermal data in calculating the energy change. As shown in table 3 the results of the colorimetric, potentiometric and thermal methods agree remarkably well.

Wurmser and Mayer-Reich (1933), Barron and Hastings (1933) and Baumberger, Jürgensen and Bardwell (1933) have applied the same principles of measurement to the lactate-pyruvate system. The agreement in the results of the first two investigations is encouraging.

In each of these cases and also (as an example from another category) in the reversible formation of the amino acid, aspartic acid, from fumarate and ammonium ion, the thermodynamic data reveal each enzyme concerned to be displaying the properties of a perfect catalyst. (See Borsook and Huffman, 1933.) Therefore each "enzyme" is presumably a reversible system. The enzyme system hastens the attainment of equilibrium from "either side." Therefore (in the first two

TABLE 3

Thermodynamic quantities:

F = The faraday E_h = Electrode potential (referred to hydrogen standard)
F = Free energy   T = Temperature (absolute)
H = Heat content   P = Pressure
U = Total energy   V = Volume
S = Entropy   n = number of electrochemical equivalents.

$\triangle$ read; "increase of"

$nFE_h = -\Delta F$ in volt faradays   1 volt faraday = 23062.8 mean calories

$$\left.\begin{aligned}\Delta F &= \Delta U + P\Delta V - T\Delta S\\ \Delta H &= \Delta U + P\Delta V\end{aligned}\right\}\text{ For reactions at constant temperature and pressure}$$

Oxidation of succinate to fumarate

$$\text{Succinate ion}^{=}(1M) \longrightarrow \text{Fumarate ion}^{=}(1M) + 2H^{+}(1M) + 2\epsilon$$

$$-\Delta F = -20{,}140 \text{ calories}$$
$$= -84{,}270 \text{ volt coulombs}$$

Heat Change (Difference in heats of combustion)

$$\text{Succinate ion}^{=}(1M) \longrightarrow \text{Fumarate ion}^{=}(1M);\ -\Delta H = -29{,}800 \text{ calories}$$

Data reduced to 25°C. basis by Borsook and Schott

INVESTIGATORS	METHOD	$-\Delta F$	$-\Delta H$
		calories	*calories*
Quastel and Whetham (1924)	Equilibration with methylene blue*	−20,180	
Thunberg (1925–1928)	Equilibration with methylene blue*	−20,100	
Lehmann (1929–1930)	Potentiometric with mediators	−20,180	−29,850
Borsook and Schott (1931)	Potentiometric with mediators	−20,140	
Borsook and Schott (1931)	Thermal	−20,460	−29,800

* Data for methylene blue by Clark, Cohen and Gibbs (1925).

cases) it presumably draws into the oxidation-reduction continuum *both* the oxidant and the reductant of the substrate. If so, the present nomenclature of enzymes may be unfortunate in its placement of *emphasis* upon activation of the reductant; upon activation of the hydrogen of the reductant. The peculiar circumstances which led to this emphasis will be made clear presently.

The cases cited have also the following significance. So little is known about the mechanism by which electromotive activity is displayed that, except for a few empirical rules, we have to depend upon luck in extending the applicability of the electrode method. The extension by the combined use of enzyme and mediator opens a new field of inquiry.

By use of the temperature coefficient of the electrode potentials there was calculated the heat change, ΔH, in the oxidation of succinate to fumarate. This is the difference in heats of combustion at constant pressure. As table 3 shows there was found a good agreement with the value obtained from the thermal data. This case and also the case of the lactate-pyruvate system furnish two more of the numerous instances in which there is a distinct difference between the free energy of the reaction, ΔF, and the heat change, ΔH. In certain important discussions of intermediate metabolism it is the change in free energy which is desired. Yet, for lack of data, treatments have had to proceed as if there were identity between ΔF and the available values of ΔH.

The potentiometric method which has been under consideration is only a special means—sometimes available and sometimes not, sometimes of the highest accuracy and sometimes not—of obtaining data upon changes of free energy and heat content. I think it fairly may be said that the potentiometric explorations have been a stimulus to more work upon the thermal measurement of the free energies of biologically important compounds—data for which we have pressing need.

To avoid possible misunderstanding I have not placed in figure 13 theoretical electrode potentials which have not been observed directly but which may be estimated from the scant, thermal data for several systems. In a few instances, where such data permit the translation, the placements in figure 13 will indicate again the tendency of metabolic systems to be of low characteristic potential. That there may be no misunderstanding of this statement let me remind you again that the electrode potential is a function of the chemical potentials which persist in the absence of the configuration of the electric cell and that it is this function which determines the *possibility* of a reaction between the given system and another whose characteristic theoretical electrode potential is known.

Furthermore, in such data as those reported by Wurmser and Geloso (1928–1931) are indications of transitory systems formed from sugars. To these also have been assigned rather negative characteristic potentials.

Diverse as are the sources of the materials mentioned in figure 13 and quite inadequate as are these and other, thermal data for many of the specific and general purposes which can be foreseen, the developments seem to be leading toward the following tentative picture.

Those cell catalysts which have been emphasized by the theoretical work of Wieland and which have been studied particularly well by the technique of Thunberg mobilize reserve food and intermediate products of metabolism from states of passive resistance. Since many of the systems[23] of which these metabolites are components have characteristic, theoretical electrode potentials which are low on the scale, their activation results in a predominantly reductive tendency toward the usual reagents. This is the circumstance which has led to the emphasis upon "hydrogen activation" by these enzymes. To the extent to which the activated systems of inherently low potential couple with the highly diluted electromotively active systems of the living cell they may affect an electrode. The tendency of an electrode or indicator to reveal very negative electrode potentials in an anaerobic culture is doubtless a crude reflection of this activation of metabolites.

Other cell catalysts "activate" oxygen and transmit its action through the oxidation-reduction continuum. Through the work of Warburg and others, several of these catalysts are becoming identified as hemochromogens. While identity is not yet complete there is reason to believe that these as well as several pigments which display supplementary respiratory functions are electromotively active. This property permits their study as components of reversible, oxidation-reduction systems. By "activation" of oxygen there cannot be meant full release of oxygen's inherent ability for then there would be no withstanding oxygen's destructive action. However, if oxygen can enter only as it converts the catalyst system to the oxidized component it is restrained.[24] Then the characteristic potential of the most positive of those catalyst systems which are in play should set a somewhat indefinite upper limit of a living cell's potential occasioned by the entrance of oxygen. This may be crudely reflected in the apparent limit found by electrode and indicator measurements of vigorously aerated cells.

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[23] By "systems" is meant here only those which are directly involved in an oxidation-reduction process.

Between the extremes observed on the one hand under strict exclusion of oxygen and on the other hand under vigorous aeration are resultants of the opposing tendencies.

This is little more than an impressionistic sketch having few of the exact delineations which are desired. Indeed, if we may judge from experience with similar and less complicated subjects, half a century will not be too long for the cautious and careful delineation which will bring out the true perspective.

In the meantime there is less profit in those speculations which give promise of the occasional practical application than in squarely facing fundamental difficulties. Some of these are: the heterogeneity of the living cell which places restrictions upon the application of data for homogeneous systems; the peculiarities of the passive resistance of molecular oxygen and possibly of several sulfur compounds; that general disparity among the passive resistances to reaction which is displayed among metabolic systems and which necessitates caution in the application of data for potential energies; the fact that timed release of passive resistance plays a rôle in behavior and may determine one of several reactions which are thermodynamically possible. We are also in distressing ignorance of conditions which determine electromotive activity and enzyme activation.

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[24] The peculiarities of oxygen have appeared in striking anomalies since quantitative measures of various equilibrium states have been made. The system hemoglobin-oxyhemoglobin displays no such electromotive activity as distinguishes the hemoglobin-methemoglobin system. The entrance of oxygen displaces the observed potentials of the latter system by removal of hemoglobin to form oxyhemoglobin but its entrance into the oxidation-reduction continuum is obviously not complete and intimate. In short oxygen here displays an important residuum of passive resistance.

Cannan (1926) notes that hermidin occurs in the plant in the reduced state, yet near places where oxygen is being evolved by the photosynthetic processes.

Such difficulties make all the more important the coöperation of skills of different kinds to obtain the sorts of data that can be integrated step by step as definite advances are made.

At several points I have departed from my theme to touch but not to trespass upon the foreign field of mechanism. What I then attempted was only to show the sort of contribution which a thermodynamic treatment may make. In the application of thermodynamics itself there are usually mechanistic assumptions which I have not paused to point out but which the student of mechanism will wish to review carefully. In this field there remains to be considered a problem the nature of which is best shown by some recent investigations of Barron and his collaborators (Barron et al., 1930–33).

In the first place Barron claims that the rapidity with which a leuco dye is oxidized by oxygen depends upon the characteristic electrode potential of the dye system. Reductants of the "positive" indophenols are oxidized slowly; other leuco dyes are oxidized the faster the more negative their characteristic potentials. One may recognize some such order without being convinced that the existing data are adequate for the theoretical use which Barron cautiously proposes. Since no well established thermodynamic principle would be violated were a usual order of this kind found not to hold in all cases, there is room for dodging and it seems to me to be preferable to avoid, for the present, theoretical entanglements and to regard the *observations* as important. It is essentially on this basis that Barron has proceeded with the idea that a supplementary cell catalyst for the oxygenative process may be a reversible oxidation-reduction system fulfilling the following specifications. The system must have a characteristic potential so positive relative to that brought about by the activation of metabolities that it can be reduced readily, yet sufficiently negative to conform to the empirical rule of easy oxidation by molecular oxygen. Systems such as those of which the indophenols are the oxidants are too positive; the safranine systems are too negative. Systems with potentials like that which characterizes methylene blue fulfill the golden mean and are effective as supplementary catalysts for oxygen consumption by starfish eggs as shown in figure 14. Especially suggestive is Barron and Hastings' (1933) use of hemin. Finding that hemin itself exerts but little catalytic activity, in the circumstances noted in figure 15, they formed the nicotine hemochromogen thereby raising the characteristic potential nearer the empirically determined optimum and obtained a striking enhancement of activity.

These empirically established relations, certain implications in Conant's (1926) extensive study of "irreversible" systems, the special case noted by LaMer and Temple (1929) and other instances of relations between potentials and kinetics are leading to an issue which may be stated as follows.

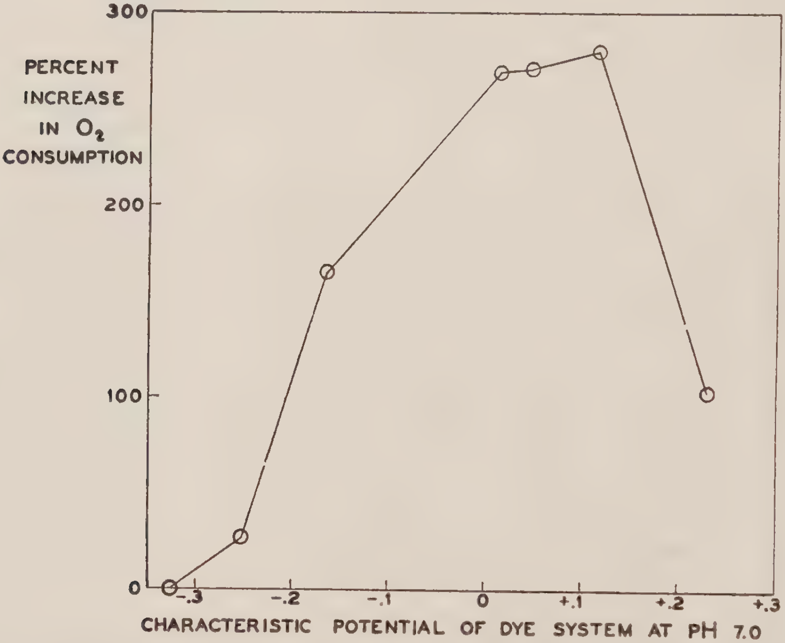

FIG. 14. RELATION OF INCREASE IN O_2 CONSUMPTION BY STARFISH EGGS TO THE CHARACTERISTIC POTENTIALS OF DYES USED AS SUPPLEMENTARY CATALYSTS
After Barron and Hoffman, 1930

It is held that a measure of a potential energy has no more to do with a kinetic event than the gravitational potential of a stone upon a shelf has to do with the release of the restraint, with the mechanisms by which the potential energy is put on display or the kinetics of this display. As Wieland put the matter in his Yale lectures (Wieland, 1932) the free energy of a chemical reaction may tell us whether the reaction can be spontaneous or not but "kinetics do not follow upon thermodynamics at all." We might wish to leave the matter there, for by so doing we should not have to face theoretical difficulties and their technical attack; difficulties which seem to be coming definitely into view. Here and there in the field of general chemistry and particularly in the field of biochemistry are found suggestions, not of a return to the abandoned theory that the free energy change determines the rate of a reaction, but of relations more subtle. To meet the challenges of existing empirical relations theory and technique will have to be strengthened and they will have to be sharpened to pierce the artefacts occasioned by the numerous, possible origins of statistical relations.

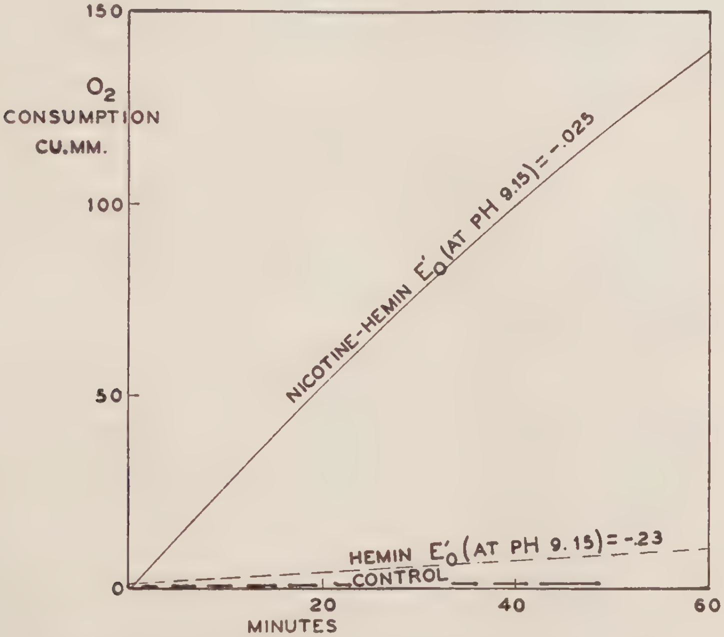

FIG. 15. OXIDATION OF LACTATE BY O_2. LACTATE ACTIVATED BY HEATED GONOCOCCI; O_2 ACTIVATED BY HEMIN OR NICOTINE HEMOCHROMOGEN
After Barron and Hastings, 1933

If I have indulged in speculation I have done so with no thought of defending any hypothesis but only to indicate the natures of developing problems. Indeed speculation would be inconsistent with the spirit of the subject which is to advance only with an artistic harmony between systematically organized, *quantitative* relations and theory adequate to their formulation. There appears evidence of a real service that a new technique can give and if some of the views I have suggested are destined to be abandoned there still will remain the imperative of the potential, defining what is possible and what is not. This should save us from false steps while we try to follow Nature's devious course in the oxidation-reduction continuum.

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EMPHYSEMA

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INTRODUCTION

Emphysema of the lungs may be defined as a condition in which the alveoli are distended, with thinning or rupture and loss of elasticity of the alveolar walls. Two forms are recognized. The first, which is characterized by voluminous lungs, and which will be referred to hereafter as "obstructive emphysema," has also received such designations as "hypertrophic," "inspiratory," "expiratory," "compensatory," and "essential" emphysema.

In the second type, the lungs are not particularly enlarged, although there may be a loss of elasticity. Clinically, this has been termed "senile emphysema." Very recently, however, it has been shown that although degenerative processes may contribute to the lesion, the essential etiological factor is a thoracic deformity from disease of the spine which leads to a barrel chest. This occurs usually in the latter decades of life, but it may appear at any age. Consequently, the term "postural emphysema" is suggested for this type.

Obstructive emphysema is by far the more important form in that it is responsible for the majority of symptoms. The greater part of this monograph will be devoted to it.

Many excellent reviews of emphysema, mostly in German, have appeared in recent years, but each has a different interpretation concerning etiology. Ever since the disease was recognized as a clinical entity by Laennec, there has been discussion over its cause; a debate which is as yet not settled. Recent investigations, however, have contributed much toward a solution of the problem.

HISTORICAL

The word "emphysema" is derived from the Greek, and translated, means inflation. It was used in Greek medical literature to denote distention of the abdomen and flatulence. Robert Lovel (1661) in his famous "Panzoologicominralogia" used the term to refer to a swelling of external wounds in which air was believed to have entered. The word then came into general medical use to designate accumulation of air within the tissues. In 1764, W. Watson reported a case of asthma upon which an autopsy had been performed, and stated that the lungs were "truly emphysematous." He believed that air was pressed into the interstitial tissue of the lung. This contention that emphysema is an extravasation of the air into the pulmonary tissues never gained ground, but the term "emphysema of the lungs" gradually came to refer to lung distention. Later, the words "traumatic" and "surgical" emphysema were introduced to indicate subcutaneous emphysema.

It is, perhaps, surprising that such a common disease as pulmonary emphysema, with its prominent chest deformity, was not recognized until comparatively late in medical history. Valsalva and other anatomists of the eighteenth century, notably Bonet, Ruysch, and Morgagni described voluminous lungs discovered at autopsy, but there was little correlation between their findings and the clinical histories of their cases. Likewise, Floyer in his "Treatise on Asthma" (1698) gave a post-mortem account of what appears to have been pulmonary emphysema.

The first deliberate study of the disease was reported by Laennec in 1819. He was not far from the truth when he stated that emphysema of the lungs such as he described seemed a disease hitherto unknown. His account of the clinical and pathological manifestations of emphysema is remarkably complete and includes both the vesicular and interlobular forms. The differentiation of these two types was based on sections of dried lungs cut very thin. Laennec, moreover, was the first to expound a theory of the mechanism underlying the production of the disease, although Floyer, and Watson previously had made suggestions concerning its etiology.

In the absence of modern methods of study and experimentation, Laennec's observations soon became widely debated and many theories concerning the cause of emphysema then developed. These were based almost entirely on clinical observations and gross anatomical material until 1861 when Rokitansky described the microscopical appearance of emphysematous lungs. A new method of study was thus introduced, although observations of unstained sections had been made by Rainey some years previously. Brown-Sequard in 1885 was the first to report the results of physiological studies on the production of emphysema in experimental animals. Since then much constructive work with physiological methods has been carried out.

INCIDENCE

There are few statistics concerning the relative frequency of emphysema. It is generally conceded that the disease occurs more often in men, due, no doubt, to greater exposure to bronchial lesions. Fränkel noted it in something over 5 per cent of 911 autopsies. According to Staehelin, the incidence at the Basle Medical Clinic from 1908 to 1912 was 2.1 per cent and from 1923 to 1927 was 2.4 per cent. Emphysema appears to be common in the tropics. Ellis found 43 cases in 100 consecutive autopsies done in Siam, and Castellani and Chambers also commented upon its frequency in the tropics. Kountz noted a high incidence in Egypt. The reason for this distribution has not been adequately explained.

ANATOMICAL CHANGES

Gross pathology

In obstructive emphysema the chief anatomical findings are distention of the lungs and their failure to collapse fully when exposed to atmospheric pressure. At autopsy, lungs from cases of extreme emphysema may appear even larger than the thoracic cavity. The margins usually approximate each other, and the heart may thus be completely obstructed from view. The antero-posterior diameter is increased and has been found to measure as much as five and one-half times the normal collapsed lung. The vertical and lateral diameters may likewise be greatly increased. Although there is general agreement that emphysematous lungs are heavier than normal, it is difficult to estimate the actual weight inasmuch as the contained secretions cannot be eliminated.

The form of the lungs in emphysema is dependent upon the size and shape of the chest cavity. The normal organ is cone-shaped with a sharp apex above, and a rounded contour anteriorly and posteriorly, below. In lungs with acute emphysema, and in early chronic cases where the chest cavity is normal, no distortion occurs. In chronic emphysema, particularly where the chest cavity is deformed by a compensatory kyphosis, the morphology of the lung changes. The portion extending from the apex to the sternum does not have a steep descent, but a very gradual slope. In extreme examples of this kind, the part under the upper portion of the sternum may be the highest point. From a side view, the posterior aspect of the lung appears more or less rounded, corresponding to the curve of the spine. The antero-posterior diameter is greatest below the sternal angle. The upper lobe is often proportionately larger than the lower, depending upon the relationship of the maximum curvature of the spine to the deformed chest cavity. The anterior and lateral surfaces are relatively flat. In conditions where the spine is straight the lobes are of more equal size as in the normal lung.

In emphysema the pleural surfaces of the lungs are stretched, smooth, and shiny. The underlying lobular markings stand out distinctly as grayish patches. Adhesions are common. Kountz and Alexander reviewing 100 cases of emphysema in the literature, found that absolute obliteration of the pleural cavity occurred in 25 per cent, partial obliteration in 45 per cent, and no adhesions in 35 per cent. They noted about the same proportions in cases other than emphysema in the autopsy services at the Barnes and St. Louis City Hospitals in St. Louis. Adhesions are due evidently to pulmonary infection and not primarily to emphysema.

Pigmented areas are found to some extent over the entire lung surfaces, but more especially at the apices and costal spaces where in advanced cases, emphysema is more pronounced. Loeschke believes that the lessened activity of the areas of lungs within the intercostal spaces is the factor which causes pigment to accumulate at these sites. Rib markings may be seen on the surfaces of the dilated lungs. These give a corrugated effect, with depressions which vary from a few millimeters to one-half centimeter in depth. They are usually most prominent in the postero-lateral aspect. In the median plane the cardiac outline and the imprint of the spinal column may appear.

Miller has pointed out the distinction between blebs and bullae appearing beneath the pleural surface. A bulla is due to vesicular

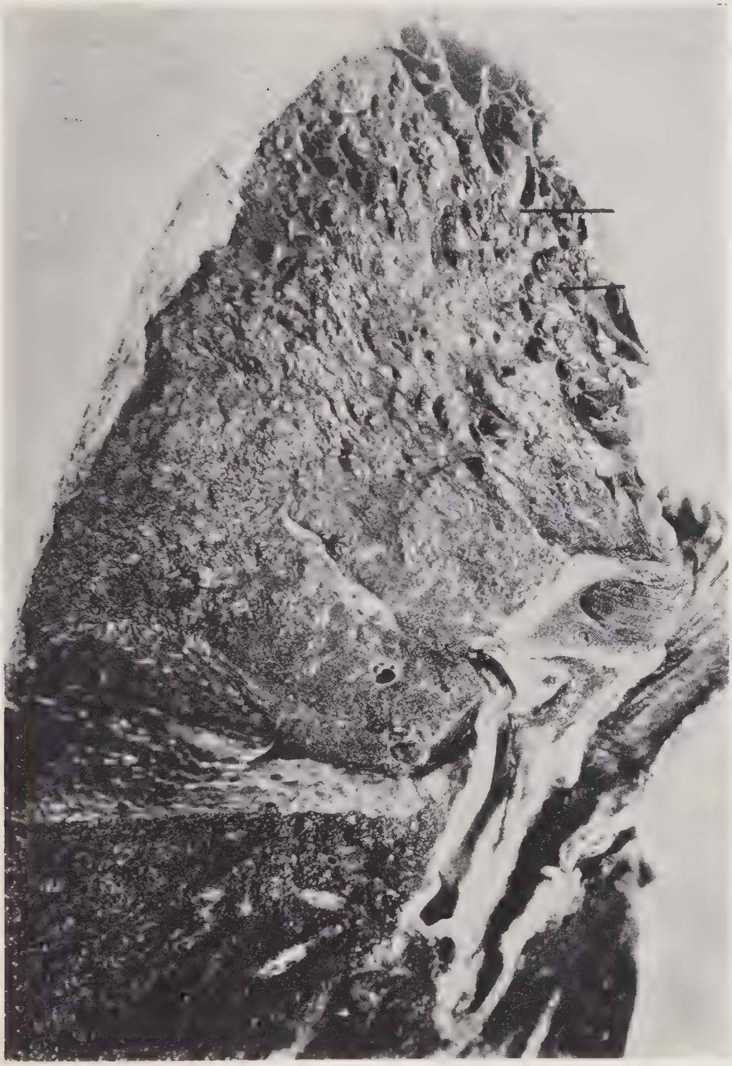


FIG. 1. CROSS-SECTION THROUGH AN EMPHYSEMATOUS LUNG

The emphysema is present throughout the entire organ but is far more extensive at the apex. (The specimen was injected with formalin and fixed before sectioning.)

emphysema situated within the lung, and although it may project, it is covered by intact pleura. Pressure upon a bulla will cause it to empty into a bronchus and collapse, provided the bronchus is not obliterated. A bleb, on the other hand, is caused by rupture of pulmonary alveoli immediately beneath the pleura. Air is allowed to escape, which then separates the pleura from the contiguous alveolar walls. Pressure on a bleb will cause it to shift position but not empty. Bullae and blebs occur in emphysema extensively at the lung apices and along the margins. They may be seen in other positions, but not commonly.

Localization of emphysema. In chronic obstructive emphysema the changes in the lungs appear more prominent at the apices and lung margins, particularly in the upper lobes. Tendeloo and Podkaminsky believed that the peripheral parts of the lung are chiefly affected, with very little change in the central portion. On the other hand, Laennec, Rokitansky, and Virchow contended that emphysema is a generalized process throughout a lobe and not localized to the periphery. Likewise, Loeschke, working with lungs into which formaldehyde had been injected before cutting them, showed that the emphysematous process extends throughout the central portion of the organ as well as at the periphery. He attributed the observations of those who reported otherwise, to faulty technic in cutting the lungs before they had been properly hardened. Localization of emphysema to one lung, and even to one lobe, have been reported in asthma, bronchiectasis, tumors obstructing expiration through an upper lobe bronchus, and in certain chest deformities.

Microscopic changes

Alveolar walls. Microscopic findings observed in emphysematous lungs are destructive in nature. The alveolar walls are dilated, stretched, thin, and many are ruptured. The capillaries are narrowed and often broken. These changes vary with the degree of bronchial obstruction and also with the duration of the disease. In the early stages such as seen in acute emphysema, following croup in children, in laryngeal stenosis, in broncho-pneumonia from influenza, and in artificially produced emphysema in animals, the position of the dilated alveoli are along the main bronchi and on the superior surfaces of the interlobular septa. As the disease becomes chronic more generalized changes occur throughout the organs. The alveoli in the apices of the upper lobes enlarge due to the transmission of increased expiratory pressure and lack of protection by supporting bony framework. The force exerted, from the bases of the lungs particularly, drives air into the upper lung spaces.

As the alveoli distend, the walls become thin, and small openings or windows begin to appear in them. These were believed by von Hansemann to be the pores of Cohn. The openings increase in size at the expense of the wall, which is reduced in thickness and takes the appearance of a thread. As the process continues, many of the septa break and one large cavity is formed. These cavities may vary from a few millimeters in diameter to the size of one's fist or larger. The only remnants of the former alveolar walls that remain are tiny broken threads attached to the inner lining of the cavity.

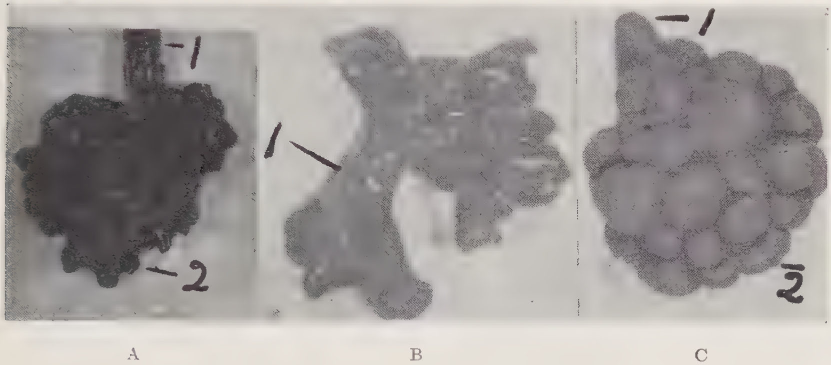


Fig. 2. Corrosion Specimens of Lung Lobules

A, normal lung; *B* and *C*, emphysematous lungs. *1* = bronchiole; *2* = alveoli

Aigner and Miller denied the existence of the pores of Cohn and believed that the observations of von Hansemann were perhaps tears in the alveolar walls. Oeritil has studied the pores of Cohn in a group of mammalian lungs, and found definite evidence of their existence in the bat and the cat. From this and other data, it was believed by Oeritil and Loeschke that the pores of Cohn do exist in the human lung although they were thought to be much smaller than in animals. The recent work of Van Allen likewise indicates an intra-alveolar communication. It has not been definitely demonstrated that these holes are the starting point of the tearing of the alveolar septa, although such a mechanism is probable.

The general form of the air sac in emphysema is much altered. The change in shape of the acini has been described by numerous observers. Miller reconstructed a portion of an emphysematous lung from serial sections and described markedly distorted air chambers. Loeschke and Kountz and Alexander injected celloidin material into a bronchus and digested the organic material away by fumes of nitric acid. The latter method leaves a cast of the air sac which may be studied with regard to its shape, size, and its relation to the surrounding alveoli. The air sacs are greatly increased in size and may stretch to many times that of a normal lung.

Bronchi. The terminal bronchioles with their alveolar ducts are also dilated. They are funnel-shaped, and lose the normal sudden enlargement of the alveolus. The dilatation extends back to the bronchi, of about 2 mm. in diameter. The air sacs of a lobule are frequently confluent in the center, and only a depression on the surface marks the outline of a former alveolus. Many lobules and air sacs within a lobule may be fused together at points where there are no normal attachments. There is no definite uniformity in shape of the small lobules and all manner of bizarre forms may be seen. Loeschke described a sausage-shaped appearance, and club forms and spindle forms occur. The normal lobule presents a picture resembling a bunch of grapes in which the alveoli extend in clusters from the ends of the small stems, which are the ducts. In emphysema, on the other hand, the stems may be dilated to the size of the alveoli. Other portions of the bronchial tree may vary. Changes in the shape of the chest from kyphosis lead to shortening of the bronchus to the upper lobe. It may be bent backward upon itself and the lumen partially obstructed. At the same time the bronchi to the lower lobes may be elongated.

The thickness of the bronchial musculature in emphysema is in dispute. Harkavy found evidence of muscle tissue hypertrophy. He believed this to be due to the ability of the muscles of the terminal respiratory passage to assist actively in the act of expiration. He looked upon muscle thickness as a work hypertrophy. The same anatomical finding has been observed by many in bronchial asthma. Huber and Koessler and Kountz and Alexander, who measured the diameter of the bronchial wall and muscle thickness in post-mortem specimens from cases of bronchial asthma with emphysema, found each to be thickened. Similar observations have been made in chronic bronchitis. Marchand noted the same thing in emphysema with bronchiectasis (bronchitis).

Muscle hypertrophy and narrowing of the bronchi are not universal findings. Marchand noted atrophy and Faschingbauer observed bronchial dilatation. All of these conditions have been found in the same lung. It should be borne in mind, however, that the configuration of a microscopic section from a bronchus may vary, depending upon the level at which the specimen is taken. In obstructive emphysema that is advanced, the terminal bronchioles and alveolar ducts are characteristically dilated, whereas the bronchi whose diameters vary between 3 mm. and 8 mm. usually show muscle hypertrophy and narrowed lumens. A distinct exception to this has been observed in a few cases associated with long standing bronchitis.

In these instances, atrophic changes in the bronchi are seen. The lesions resemble those of bronchiolitis obliterans. The smaller bronchi may be obliterated by chronic inflammatory tissue. In some of the larger ones there appears to be a concentric ingrowth of inflammatory elements toward the lumen of the bronchus, while in others it may occur more on one side. All the bronchial structures show atrophic changes. The muscle is thinned and in serial section appears patchy. It does not completely encircle the bronchus. The glands, less numerous than normal, are atrophic although some exudate may be present in the ducts. The epithelium is low cuboidal and atrophic. The subepithelial layer may be increased in thickness and infiltrated with lymphocytes and polymorphonuclear leucocytes. Usually, however, it is thin and has the same general wasted appearance as the other elements. The bronchi are irregularly dilated especially those whose calibre is about 6 mm. to 8 mm. The general appearance of the lungs indicates that a chronic inflammatory process has occurred with an overgrowth of granulation tissue. The alveoli are dilated and torn and loss of elastic tissue may be definitely demonstrated by elastic tissue stains.

The question of change in elastic tissue in emphysema is in dispute. Grawitz demonstrated a congenital defect in the formation of elastic fibers in emphysema. His work was supported by Bayer and Greenow. Tendeloo, on the other hand, who carefully studied the problem, maintained that the elastic tissue is only stretched and thus apparently, but not actually diminished.

In acute emphysema observed in patients who died from influenzal pneumonia, from whooping cough, and from bronchial stenosis McCordock found elastic tissues to be normal or actually increased. Likewise, in the emphysematous lungs of dogs with the trachea obstructed, we observed no change in the amount of elastic tissue. In the chronic cases with very large lungs, however, our impression is that there is an actual decrease in elastic tissue fibers which frequently appear to be stretched and broken.

Much attention has been directed to the pulmonary vessels in emphysema, particularly since its etiology has repeatedly been attributed to disease of the pulmonary arteries. It is generally agreed by pathologists that as emphysema progresses there is a gradual obliteration of the pulmonary bed. Virchow early pointed out that many of the capillaries are broken and occluded. Even though the capillaries are not ruptured when the bronchial walls are stretched, the vessels are narrowed and the lungs become anemic. Hemorrhage may occasionally occur in the alveolar spaces. The obliterative arteritis may extend to vessels larger than the terminal capillaries, and in advanced cases, arterioles even several millimeters in diameter may show sclerosis.

A change in the epithelial element of the lung in emphysema consists of fatty and mucoid degeneration of the lining cells of the alveoli and ducts. In addition, desquamation and denudation have been described. It is noteworthy, however, that Tichmenoff found the bronchial epithelium to be hypertrophic in some cases of emphysema associated with asthma.

Exudate in the bronchi from increased secretion of the bronchial mucous glands has been found in practically all cases of asthma with emphysema that have come to autopsy.

The lymph vessels of the lung in emphysema have never been investigated. Pathological observations lead one to believe that these suffer the same fate as the blood vessels. Microscopically the pleural lymph channels may be seen to be infiltrated with leucocytes and wandering cells. Accumulation of pigment and cells in the periphery of the lung suggest that lymph vessels leading to the hilus are obstructed.

Pronounced changes in the size and shape of the lungs in emphysema are reflected in a corresponding alteration of the thoracic cage. As will be pointed out, the mechanism by which an emphysematous individual curves his spine is a compensatory one. This factor serves to distinguish the spine of obstructive emphysema from the postural type. In the former condition the entire thoracic spine takes part in a slow graceful curve, while in the latter localized changes occur. The bodies of the vertebrae in obstructive emphysema usually show some lipping in their anterior portion. Posteriorly there is usually no change. The anterior tips of the intervertebral discs may be eroded and compressed, and in some instances the tips of the vertebral bodies come in contact with one another.

Acute vesicular emphysema may lead to collapse of the lung from a pneumothorax caused by rupture of a bulla on the surface. At autopsy, if the lung is subjected to pressure, the opening in the bleb may be found. Interstitial emphysema also occurs. This is due to localization of the dilated air sacs around the large bronchi, and along the superior surfaces of the lower and middle lobes. The alveolar walls rupture and air spreads along the pleura to the mediastinum, and mediastinal emphysema is thus produced. Siems noted the formation of vesicular and interstitial emphysema in patients with bronchopneumonia following influenza. He found air mainly in the anterior and posterior mediastinum, and in the retroperitoneal tissues. Wentzler examined two cases of generalized subcutaneous emphysema and found it to result from rupture of alveoli. The air spread along the bronchi and trachea to the mediastinum, and then to the subcutaneous tissues of the neck. Siems determined the route the air took by blowing up the lungs with a pump. He observed that it traveled via the root of the lung, and then followed the reflection of the pleura and pericardium over the great vessels. Kountz, Alexander and Dowell noted that when expiration was obstructed to a considerable extent in dogs by a valve in the trachea, a generalized interstitial emphysema resulted. The route was along the root of the lung and followed the reflection of the pleura into the neck, and then spread over the body. Occasionally in these animals it appeared below the diaphragm into the retroperitoneal tissues. The subcutaneous spread, however, was always by way of the neck to the posterior portion of the body.

Changes in the circulatory system will be considered in another section.

RESPIRATORY CHANGES

Muscle action. In emphysema, respiratory movements are profoundly affected, due in part to impairment of muscle activity. Both the diaphragm and the intercostals are involved.

In normal individuals, the action of the diaphragm accounts for 40 per cent of the vital capacity of the lungs. In ordinary quiet breathing, this muscle descends about 1.2 cm., whereas on deep inspiration its excursion may be 3 cm. to 6 cm., depending upon the shape of the chest. In obstructive emphysema, the diaphragm is gradually pushed downward as the lungs increase in size, so that its action becomes more and more limited. In advanced cases the position of extreme contraction may be reached, and it then can no longer function. Under this condition, the intercostal and accessory muscles take up the entire burden of respiration. As they expand the chest, occasionally the diaphragm actually ascends during inspiration as it is drawn upwards by the thoracic cage. This paradoxical movement may be distinctly observed under the fluoroscope.

The intercostal muscles are likewise impaired in obstructive emphysema, for as the chest assumes a barrel shape the ribs separate and the levators of the ribs are distorted. Creuyx stimulated, electrically, the nerves to these muscles in cases of emphysema, and maintained that he demonstrated a deficiency of their action.

In recent years, a considerable amount of data has been assembled to indicate that the muscles of the bronchi actively assist in the process of respiration. There is some dispute as to whether contraction of the bronchial musculature occurs during inspiration or expiration. Hudson and Jarre, however, by taking cinema x-ray pictures of lungs into which lipiodol had been injected, confirmed Miller's prediction that the expiratory phase is affected. They observed peristaltic movements in the bronchi as the air left the lungs. There is increasing evidence which points to the probability that contraction of the bronchial musculature plays a very important rôle in obstructive emphysema. This was first suggested by Brown-Sequard, who induced bronchospasm in dogs and rabbits by electrically stimulating the midbrain near the vagus nucleus. The lungs were exposed during the experiment, and as the bronchi contracted the alveoli were seen to dilate. Brown-Sequard naturally assumed that some of the air in the bronchial space was squeezed backward into the alveoli. These experiments were confirmed by Kountz and Alexander, who recorded changes in alveolar pressure on a manometer. Also Jagic and Spengler called attention to the fact that clinical improvement of emphysema may be secured by administration of adrenalin, but apparently little attention has been paid to this observation.

A few patients with advanced emphysema in whom we recently studied the effect of adrenalin on vital capacity, showed a striking improvement in their symptoms. In one case the vital capacity rose from 1100 cc. to 2900 cc. within ten minutes. In another it rose from 1300 cc. to 2800 cc. in seven minutes. The effect could be prolonged to a considerable degree with ephedrin. These patients were subject to paroxysmal attacks of dyspnea, and although some distant wheezing could be discerned on auscultation at such times, rhonchi and musical râles were absent. It is noteworthy that in one of these patients no history of asthma, bronchitis or other causes of bronchial obstruction could be elicited, and yet the patient exhibited all the signs of advanced emphysema. This is the type of case which has thrown doubt on the contention that obstruction to breathing is an immediate cause of the disease. From these observations, it would seem quite probable that an undetected bronchospasm may lead to advanced emphysema. No opportunity has been afforded as yet to measure in such cases the thickness of the bronchial musculature, hypertrophy of which would support this contention.

Intrapleural pressure. At birth, the lungs are of the same size as the chest cavity and contain very little elastic tissue. The intrapleural pressure is atmospheric. Pulmonary excursion is then dependent upon the adhesive forces between the moistened pleural surfaces. As growth occurs, elastic tissue increases, which tends to keep the lungs smaller than the thoracic space and permits the development of a negative intrapleural pressure.

If diaphragmatic movement is unimpaired, deep inspiration causes the pleural pressure to become more negative, and in extreme conditions values as low as −70 cm. of water have been recorded. Conversely, during expiration the intrapleural pressure rises, and if this force be exaggerated as in coughing or other conditions where bronchial obstruction impedes expulsion of air, the pressure may rise far above the atmospheric. Emerson cited a reading of +75 cm. water.

As emphysema develops there is at first an increased negative intrapleural pressure which later becomes positive. The initial stage of the process is comparable to a normal deep inspiration, during which the lungs expand. Where no bronchial obstruction is present, the distention may, under artificial conditions, be so great that the alveoli tear and a typical picture of emphysema is then observed in microscopic sections. Prinzmetal demonstrated this change by exposing rats to abnormally reduced atmospheric pressures for long periods, thereby attaining very low intrapleural pressures associated with emphysematous lungs. Doubtless, the apparent emphysema observed at high altitudes is induced by a similar process. The acute emphysema occurring from overexertion is likewise inspiratory in origin.

In cases with bronchial obstruction, the initial process of lung expansion is associated with an increase in negative intrapleural pressure due to the inspiratory effort of drawing air past the obstruction. In expiration, if the force exerted to push the air past the obstruction is not sufficient, air accumulates in the alveoli and the lungs distend. As long as the diaphragm and the chest wall can "give," however, they absorb the pressure exerted by the enlarging lungs and intrapleural pressure remains negative. As the distention progresses and the size of the lungs comes to equal or exceed that of the thoracic cavity, there is an abrupt rise in intrapleural pressure not only during expiration, but also during the whole respiratory cycle.

This mechanism has been demonstrated experimentally by inducing anaphylactic shock in guinea pigs and thereby creating intense emphysema from bronchospasm. As the shock first became manifest by increased respiratory effort, there was a marked fall in intrapleural pressure. This represented the phase of lung distention as the animal forcibly pulled air through the constricted bronchi. Since the forces of expiration were insufficient to expel the air taken in, some of it became trapped in the alveoli with each breath. The lungs, enlarged more and more until the diaphragm was pushed down so that it could no longer act and the chest was likewise greatly distended. The muscular effort to expel air then caused an abrupt elevation in intrapleural pressure which finally exceeded atmospheric. The same thing has been observed in patients during an asthmatic attack. At the onset, while the lungs are expanding, the intrapleural pressure falls, whereas later when distention is great, it rises. Kountz, and Christie, measured the intrapleural pressure in individuals with well-established obstructive emphysema, and although it varied in different locations in a given case, it was uniformly increased. Christie has recently pointed out that as long as the intrapleural pressure remains negative, the emphysematous lungs are capable of expansion, but when distention is complete, the intrapleural pressure fluctuates around the atmospheric level.

TABLE 1

Intrapleural pressures in normal individuals and emphysematous patients

PATIENT	VITAL CAPACITY	TIDAL AIR	INTRAPLEURAL PRESSURE*	
			Expiration	Inspiration
	cc.	*cc.*	*cm. water*	*cm. water*
1. Normal	3,800	375	−4	−6
2. Normal	4,200	425	−3	−7
3. Normal	4,500	480	−4	−8
4. Emphysema	4,100	380	−2	−5
5. Emphysema; asthmatic attack	2,200	200	+2	−8
6. Emphysema; asthmatic attack	1,900	300	+4	−3
7. Emphysema	3,200	225	−1	−6
8. Emphysema	2,700	300	−2	−4
9. Emphysema	2,800	270	−1	−4
10. Emphysema (primary pulmonary arterial sclerosis)	1,700	200	+2	−8

* All intrapleural pressures taken in mid-axillary line in fifth interspace.

Another important function of the negative intrapleural pressure is to assist in drawing blood into the right heart. The disturbance of this process in emphysema, from the elevated pressure within the pleural space, has a profound effect on the circulation which will be discussed later.

The normal interbronchial pressure is higher than the intrapleural pressure. During inspiration it is from 1 cm. to 3 cm. of water less than atmospheric, and with expiration 3 cm. to 5 cm. above. Neergard and Wirz studied the relationship between the intrapleural and interbronchial pressures in both normal and emphysematous individuals. Their results are rather surprising in that in emphysema the inspiratory bronchial pressure was lowered, whereas the expiratory pressure was usually normal and not elevated.

Pulmonary ventilation. The disturbed ventilation in emphysema is due primarily to the inability of the lungs to contract properly. Tendeloo, Hennemann and Metz studied the elastic quality of normal and of emphysematous pulmonary tissue. They found that when a normal lung was stretched and the tension then released, the organ returned to its original position. Even microscopically, no distortion could be discerned. In acute emphysema, the elastic response was diminished, as was the case in senile and presenile emphysema. In the chronic obstructive form of the disease they noted a marked diminution in elastic response as well as in elastic tissue fibers.

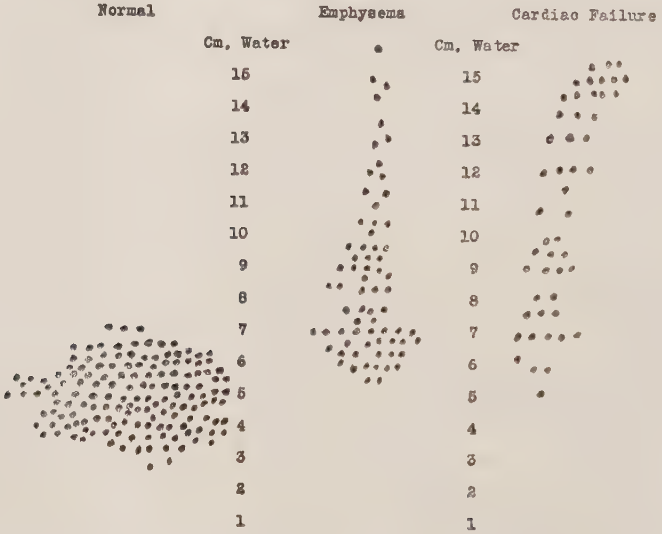


FIG. 3. A COMPARISON OF VENOUS PRESSURES OF A GROUP OF NORMAL INDIVIDUALS WITH THOSE HAVING EMPHYSEMA AND CARDIAC FAILURE

Each dot represents the venous pressure of a given individual. There are 151 normal controls, 69 cases of emphysema, and 48 of cardiac failure.

The recent investigation of this subject by Christie has yielded convincing results. He measured the pulmonary elasticity and distensibility by simultaneous registration of the intrapleural pressure and the volume of tidal air. The ratio between the two in a normal elastic lung when plotted, yields a horizontal line, the stress being proportional to the strain. In emphysema a logarithmic curve is obtained characteristic of a non-elastic body in which the same force can produce different degrees of distention; the deviation of application of the force being the variant.

In emphysema, the vital capacity is consistently reduced. Peabody found that the total volume of the lungs was not diminished but that the residual air increased at the expense of the complemental and supplemental air. The tidal air usually was normal in amount. In a given case of obstructive emphysema, the vital capacity may vary from day to day, depending probably on the type and state of the obstruction. This is particularly noticeable in patients with asthma and with bronchitis. In the series of cases in table 3 the average reduction is 43.4 per cent of the normal as estimated by Dreyer's figures.

Recently Hurtado and his associates applied the method of Christie to determine the total pulmonary capacity and its subdivisions. They then made preliminary observations by the same methods in nine cases of emphysema. In seven of these the total capacity observed corresponded closely with that predicted from measurements of the chest cavity. Increase in the volume of the residual air and a corresponding reduction in the vital capacity was observed in all cases.

According to several observers, the minute volume is increased. In Staehelin's series of 22 patients with emphysema under 50 years of age, the average volume was 9.5 liters, compared to 7.5 liters in 14 normal controls. This increase was accomplished chiefly by an accelerated rate of breathing, although the tidal air was also somewhat greater than the controls. The duration of inspiration and of expiration as well as the ratio between the two fell within the wide normal limits.

Peabody, Siebeck and others maintained that the gas exchange in emphysema is interfered with because of the marked increase in residual air. The midpoint of breathing is thus raised and becomes further removed from the pulmonary capillaries. Dreser and Beitzke contended that the faulty exchange of air in the emphysematous lung is due to a distortion of the small bronchioles. They pointed out that these are dilated and funnel-shaped in contrast to the normal uniformly narrow tube ending in a cluster of widened air sacs. These investigators made glass models of terminal bronchioles with the attached alveoli, of both normal and emphysematous lungs, and passed gases through them. They found a distinctly poorer exchange in the funnel-shaped models than in those of normal lungs. They therefore assumed that in emphysema the gaseous exchange takes place in the more proximal bronchi with a resultant elevation in the midpoint of breathing. Peabody and others have suggested that the faulty ventilation may be due to a defect in the alveolar epithelium.

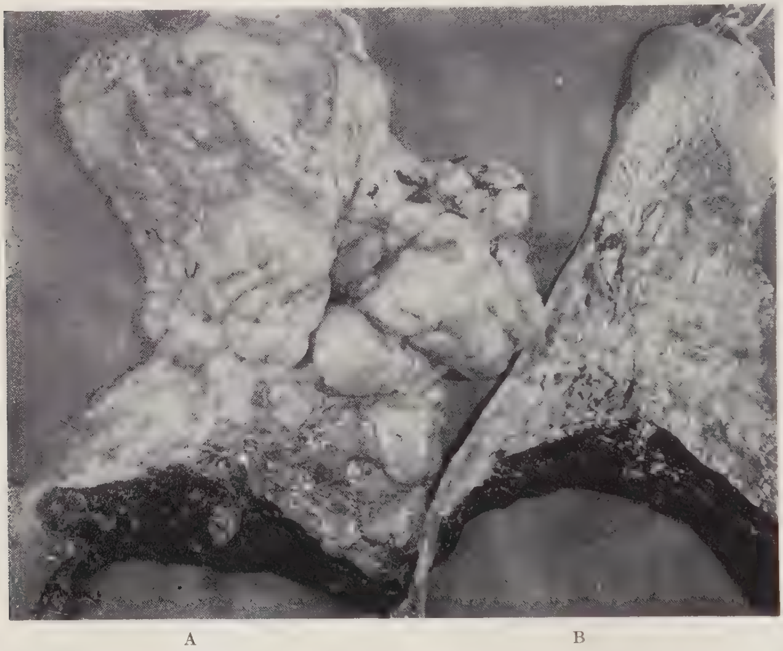


Fig. 4. *A*, upper lobe of the lung of a "wind broken" horse; *B*, upper lobe of the lung of a normal horse. Both animals were approximately the same size.

In emphysema there is a diminution in the total alveolar surface which suggests that changes may occur in the remaining alveoli to interfere with gas exchange. Haldane called attention to the stiffness of the lungs and lack of churning motion which normally aids in the diffusion of gases.

The exact mechanism of the impaired hemorespiratory exchange is not known. Christie suggested that with the muscles of respiration in the inspiratory position, and the lung inelastic, inspiratory effort is ineffective and response is limited largely to outlying alveoli, many of which are functionless.

As a result of faulty gas exchange in emphysema, the O_2 of the alveolar air is diminished and the CO_2 increased. The oxygen content of the blood is likewise reduced. Meakins and Davies, who were the first to study this, found that the saturation of arterial blood varied from 86 per cent to 90 per cent. They noted, however, that when emphysematous patients were exposed to air enriched with oxygen, the O_2 content of arterial blood assumed normal values. They believed this faulty oxygenation of the blood to be due to alveolar changes. Meakins' and Davies' observations have been confirmed by various observers, some of whom recorded considerably lower values for arterial O_2. Himwich and Loebel reported an amazing reduction in the degree of saturation of hemoglobin of the arterial blood from 78 per cent to 23 per cent in a patient with severe emphysema during slight exertion.

Scott found an elevated bicarbonate content of the blood in emphysema, and considered this a result of faulty pulmonary ventilation. The ratio $\frac{H_2CO_3}{NaHCO_3}$, however, was such as to maintain the H-ion concentration within normal limits. Peters, Bulger and Eisseman pointed out that this elevation of the carbonates in the blood is associated with a reduction in the chloride content. The alkaline reserve may be increased to twice normal, while the chlorides may drop to 87 millimols as compared to a normal value of 96. These investigators believed that the reduction of chlorides compensates for the excess carbonate electrolytes and serves to keep the H-ion concentration of the blood within normal limits.

Scott also discovered that patients with emphysema have an increased tolerance for rebreathing CO_2, a finding that has been verified by other observers. This increased tolerance for CO_2 tends to compensate for the insufficiency of pulmonary ventilation. However, it was pointed out that an emphysematous individual is incapable of responding adquately to the stimulus of CO_2 because of his limited respiratory excursion. His rate may increase, but above certain concentrations he may go into collapse.

CIRCULATORY CHANGES

The heart. It has been a time-honored belief that as a result of emphysema, the heart sooner or later fails. The mechanism of cardiac damage in these cases seemed quite obvious, for the marked distention of the lungs was thought to impede the passage of blood from the right ventricle to the left auricle. This supposition had experimental support in that the older anatomists described narrowing and destruction of pulmonary capillaries, which enter into the structure of the walls of the alveoli and which necessarily must rupture when pulmonary distention becomes sufficiently great to tear lung tissue. As a result, it has been argued that an excess load is placed upon the right ventricle in its effort to force blood through the obstructed capillary bed. This, in turn, leads to dilatation and hypertrophy of the right ventricle, and finally to heart failure. Clinically this seemed to be true, for not only were obvious signs of cardiac decompensation found commonly in elderly individuals with senile emphysema, but dyspnea, cyanosis and dependent edema were noted frequently in younger persons with pronounced emphysema. Some investigators have believed that all cases of emphysema die of right heart failure.

Quite recently new light has been shed upon this problem. To begin with, it has been shown that senile emphysema which is often associated with obvious cardiac disease is not primarily a pulmonary disorder. The cardiac manifestations are entirely independent of the pulmonary findings. It is true, however, that dyspnea, cyanosis and at times dependent edema, are associated with emphysema following asthma, chronic bronchitis, and other obstructive lesions of the bronchi. These signs point to myocardial insufficiency and have served to maintain the belief that emphysema leads to heart failure. This problem has been studied both from an experimental and a pathological approach.

Recent investigations have thrown some doubt as to whether the lung distention of emphysema offers an effective obstruction to the flow of blood through the lungs.

Kretz studied the effect of emphysema, experimentally, on the right heart of dogs. He found it difficult to obtain true values of vascular pressures within the closed thorax because of the ever-changing intrathoracic pressure which is distributed evenly upon all the thoracic viscera. He concluded, however, that sufficient evidence had not been presented to warrant the assumption that emphysema alone causes hypertrophy of the right heart.

Lichtheim attacked the problem by tying off areas of lung tissue, and found that a very considerable amount of lung had to be obstructed before a rise in pressure in the pulmonary artery could be recorded. Kountz, Pearson, and Koenig measured the circulation time through the lungs by injecting a dye into the right heart of dogs in whom emphysema had been induced. They recorded the time it took for the dye to appear in a peripheral artery and found no delay over normal dogs. Evidently, there was no obstruction to the flow of blood through emphysematous lungs.

Blumgart and Weiss, by injecting a radio-active substance into the vein of one arm and measuring the time it took to detect emanations in the other arm, found that only in far advanced cases of emphysema was there retardation in the rate of blood flow through the lungs.

Heinbecker's experiments were striking in their results. He perfused the pulmonary artery and measured the rate of flow in relation to lung distention. It could thereby be shown that when the lungs were moderately distended the flow was increased due to straightening of capillaries and the opening of new ones, but when distended to a greater extent the flow was diminished.

From the above data, it may be inferred that in clinical obstructive emphysema the enlarged lungs do not materially effect the flow of blood from the right to the left heart, unless the process is far advanced, when some retardation may occur.

Podkaminsky made X-ray observations on a group of patients with emphysema, and noted that the enlarged right ventricle spoken of as the "emphysema heart" by others did not appear in his cases. He observed the heart to be small.

Alexander and Kountz studied a series of cases at autopsy. They noted that the heart presented one of three findings. In the majority, not only was there no ventricular hypertrophy, but the heart frequently was smaller than normal. In order to obviate the uncertainty of linear measurement of cardiac muscle, the right ventricle was weighed and compared to the total heart weight according to Lewis's method. No myocardial changes were discovered.

In cases where cardiac decompensation was present, changes in the heart did occur. Almost always both the right and left ventricles were hypertrophied. This unexpected lesion is difficult to interpret, for if obstruction to the pulmonary circulation were actually sufficient to cause a work hypertrophy of the right ventricle, the left side of the heart, in a sense, should be spared. No peripheral factors, such as hypertension or arteriosclerosis, were discovered to account for left ventricular enlargement and the cause of this lesion is not definitely known. It had been observed by Hoover, who suggested that there may be a partial asphyxia of the heart muscle which would lead to increased effort and generalized hypertrophy. In this connection, it is interesting that Vacek was able to produce a generalized cardiac hypertrophy in mice exposed for varying periods to low oxygen tensions. Stronghold subjected animals to varying degrees of asphyxia and found that a reduction of O_2 in the inspired air resulted in an increase of both the diastolic volume of the heart and of the systolic discharge. Hoover's theory, however, is open to doubt for one may have a considerable degree of prolonged cyanosis in pulmonary disorders without an hypertrophied heart. Moreover, Kountz and Gruber produced cardiac asphyxia by the injection of cardiac pressor substances as well as by general asphyxia in dogs, and obtained a typical elevation of the R T segment on the electrocardiogram. No such definite curves have been noted in cases of emphysema in our patients.

Geizel explained the generalized ventricular hypertrophy in emphysema as due to increased abdominal pressure caused by the forced descent of the diaphragm. A resistance to a large part of the peripheral blood flow is thereby offered and the heart responds by more forceful systolic contraction of the left ventricle. He believed that the raised intra-abdominal pressure likewise hastens the return flow to the right heart, whose intake is no longer regulated by proper intrapleural pressure. Geizel concluded, therefore, that both ventricular chambers are at a disadvantage in emphysema, and hypertrophy of each occurs. However, Alexander and his associates have found that during pulmonary distention the effective venous pressure in the right auricle is actually decreased.

The fact that generalized cardiac hypertrophy occurs in patients with obstructive emphysema suggests that in these cases there may be an increased viscosity of the blood due perhaps to polycythemia. It is known that an increase in red blood cells occurs in some cases, but this is not the rule. The possible association of these two factors is being investigated.

A suggestion offered by Sibson and considered by older clinicians is that emphysema occurs during the process of loss of elastic property to tissue of the body. The lungs alone are not affected, but the heart and other organs as well. There is no proof for this statement but the suggestion is worth consideration, and cannot be passed over until we understand more about degenerative changes in the body.

Beside the normal heart and that in which generalized ventricular hypertrophy occurs, there is still another type of heart in emphysema. Here, there is right ventricular hypertrophy alone. Although this had been believed to be the common lesion in emphysema it was a rare finding in our series. When right ventricular hypertrophy alone was found it was usually associated with changes in the pulmonary arteries. We doubt seriously whether any marked degree of selective right heart hypertrophy does occur unless there is an associated pulmonary arteriosclerosis. However, it is possible that in the earlier stages of obstructive emphysema when the intrapleural pressure is more negative than normal, the return of blood to the right heart may be accelerated and the right ventricle thus overdistend. In the later stages, this mechanism is no longer possible since the intrapleural pressure approaches atmospheric and the flow of blood to the heart is retarded.

In the majority of cases of obstructive emphysema reported in the literature upon which postmortem studies have been made, very little evident damage to the right heart alone has been reported. In MacDonald's series, however, there were eight cases of asthma, seven of which had emphysema. In the eighth case, the lungs were recorded as larger than usual but he does not state whether emphysema was present. The average thickness of the left ventricular wall was 18.25 mm., and of the right ventricular wall 7.87 mm. This would lead one to believe that there was hypertrophy of the right heart. The report of these cases is interesting since it is a review of autopsy material and

TABLE 2

Statistics on fourteen patients with extreme emphysema who died

CASE	CLINICAL DIAGNOSIS	AGE	ELECTRO-CARDIOGRAM	SURFACE AREA OF HEART	VITAL CAPACITY	PERCENTAGE OF THE ESTIMATED NORMAL VITAL CAPACITY	ARTERIAL BLOOD PRESSURE	VENOUS BLOOD PRESSURE	EDEMA	OXYGEN CONTENT OF ARTERIAL BLOOD	PER CENT OF OXYGEN SATURATION OF ARTERIAL BLOOD	CARBON DIOXIDE CONTENT OF ARTERIAL BLOOD	RATIO OF WEIGHT OF RIGHT VENTRICLE TO LEFT VENTRICLE†
					cc.			*cc. water*		*per cent by volume*		*per cent by volume*	
1	Bronchitis	64	RVP	Normal	2,200	48	130/80	9		14	77	47	1:1.36
								12*	3+	11*	64*	50*	
2	Asthma	69	Normal	Normal	1,800	48	105/72	9.5	2+	14	82	56	1:1.40
3	Bronchitis	65	RVP	Normal	1,500	48.5	148/95	10.5	2+				1:1.48
4	Asthma	52	Normal	Normal	1,700	43	115/85	10.75	3+	11	70	52	1:1.35
5	Bronchitis	58	Normal	Normal	2,400	62	109/80	7.5	4+				1:1.38
6	Bronchitis	64	LVP	Normal	2,600	66	160/95	9.5	3+	14	70	54	1:1.52
7	Asthma	51	Normal	Normal	1,800	47	118/80	10	3+				1:1.38
8	Asthma	60	Normal	Normal	1,700	43.8	130/85	13.5	3+				1:1.80
9	Asthma	48	LVP	Large	2,000	45	120/75	14	3+				1:1.45
10	Asthma	56	RVP	Large	1,800	50	115/80	16	None				1:1.30
11	Bronchitis	58	LVP	Large	2,600	60	118/76	10	3+				1:1.28
12	Tumor of trachea	42	Normal	Normal	1,800	45	110/70	12	None				1:1.46
13	Unknown	50	Normal	Large	2,200	54	175/70	14	+				1:1.20
14	Unknown	55	Normal	Large	1,600	46	118/80	20	3+				1:1.05

* During exacerbation of symptoms.

† Normal ratio = 1:1.42 to 1:2.00. RVP = right ventricular preponderance. LVP = left ventricular preponderance.

records compiled by several observers over a period of some thirty-five years. Much data is lacking. In the first two cases, measurements of the ventricular walls suggest a left hypertrophy as well as a right. In other cases, measurements of the left side are lacking and one is unable to judge to what degree, if any, the left ventricles were affected. To arrive at accurate estimations, it is necessary to weigh the ventricles separately and to compare these with the total weight of the heart.

Clinical studies lead one to believe that the heart is not often affected. Alexander, Luten and Kountz in their series of patients with long-standing asthma and emphysema found that the heart was singularly free from damage (table 3). Criep recently has confirmed these findings. Walzer reviewed the subject and likewise agrees with this conclusion. Kahn, however, maintained that many cases of asthma show cardiac changes inasmuch as he found a tendency to right ventricular preponderance on the electrocardiogram. He reported no other clinical findings, and his observations have not been substantiated.

Inasmuch as the preponderance of evidence points to the fact that there is no cardiac lesion in the majority of cases of emphysema, the finding of dyspnea, cyanosis and dependent edema, which often occur, must be accounted for. The dyspnea is readily explained by the marked lowering of the vital capacity, which at times becomes so reduced that but slight physical exertion causes shortness of breath. The cyanosis is proportionate to the reduction of O_2 in the arterial blood and can be due entirely to faulty pulmonary ventilation. The mechanism of dependent edema is more difficult to explain but is believed to be brought about by increased venous pressure. This will be elaborated upon under that heading. The possibility is kept in mind that some cases represent early heart failure, in which the myocardial strain is not sufficiently pronounced to enable one to recognize change in the heart.

Patients with normal hearts, in whom dyspnea, cyanosis and dependent edema occur, are distinguished from those with right and left ventricular hypertrophy by orthodiagrams of the cardiac outline. In the latter cases there is a distinct increase in the cardiac diameters, particularly of the right ventricle, whereas in the former the heart is tubular and spindle-shaped. In cases of pulmonary arteriosclerosis with signs of cardiac failure, the right side of the heart may appear

TABLE 3

*Cardiac findings of 50 patients with bronchial asthma of more than 5 years standing with emphysema**

	CASE	AGE	DURATION	CARDIAC HISTORY	ENLARGEMENT	RATE	MURMURS	RHYTHM	ACCENTUATED PULMONARY SECOND SOUND	BLOOD PRESSURE		EDEMA	SURFACE AREA OF HEART	ELECTROCARDIOGRAM	VENOUS PRESSURE	EMPHYSEMA	VITAL CAPACITY	PER CENT ESTIMATED VITAL CAPACITY	IMPRESSION
										Systolic	Diastolic								
			yrs.												cm. water				
1	W. H.	28	7	0	0	60	0	R	0	108	87	0	Large	Normal	5.5	±	4	95	Normal
2	T. W.	43	15	0	±	92	0	R	0	128	76	0	Small	Normal	6.5	+	2.4	63.3	Normal
3	N. P.	33	11	Edema	±	118	Syst. base	R	0	110	65	0	Small	Normal	13.5	+	1.2	46.5	Normal
4	M. M.	33	6	0	0	96	0	R	0	120	60	0	Small	Normal	6	+	2.7	42.9	Normal
5	W. W.	40	7	Syphilis	±	96	0	R	0	114	68	0	Normal	Normal	6	+	2.1	28	Normal
6	G. D.	60	27	0	±	80	0	R	+	118	80	+	Normal	Normal	8	+	2.7	43.8	Normal
7	A. B.	57	10	0	0	70	0	R	0	134	72	0	Normal	Normal	5.5	+	2.8	60.3	Normal
8	B. H.	53	11	0	±	87	Syst. base.	R	0	156	94	±	Normal	L.V.P.	6.2	+	2	45.5	Pathologic?
9	O. A.	52	8	0	0	87	0	R	0	135	70	0	Normal	Normal	8.5	+	1.8	20	Normal
10	D. V.	46	15	0	0	95	0	R	0	115	70	0	Normal	Normal	7	+	2.2	54.9	Normal
11	L. B.	39	15	0	±	80	0	R	+	150	90	+	Normal	L.V.P.	5	+	1.7	38.4	Pathologic?
12	J. G.	50	18	0	0	78	Syst. apex	R	0	130	82	0	Normal	L.V.P.	7	+	1.7	45.6	Pathologic?
13	F. A.	32	6	Syphilis	0	80	0	R	0	170	98	0	Normal	Normal	5	+	4.2	71.7	Normal?
14	A. T.	22	11	0	±	70	0	R	0	120	80	0	Normal	Normal	6	+	2.5	57.2	Normal
15	A. A.	38	18	0	±	80	0	R	0	117	68	0	Normal	Normal	6	+	3.2	59.9	Normal
16	O. A.	51	27	0	0	90	0	R	+	106	68	0	Normal	L.V.P.	5.8	+	1.5	27	Normal
17	J. F.	78	16	0	±	90	0	Irreg.	0	135	95	0	Large	Aur. fib.	13	+	2.5	51.8	Pathologic
18	L. K.	48	6	0	±	74	0	R	0	124	70	0	Large	Normal	7.5	+	2.7	64.1	Normal
19	H. K.	54	20	0	±	80	0	R	0	132	88	0	Normal	Normal	7	+	2.0	65.6	Normal
20	S. K.	50	20	0	±	80	0	R	0	126	90	0	Normal	Normal	5.5	+	2.2	—	Normal
21	A. P.	37	5	0	±	80	0	R	0	108	68	0	Normal	Normal	5.5	+	2.2	67.3	Normal

22	M. Y.	8	7	0	±	80	Syst. base	R	0	95	70	0	Normal	Normal	7	±	2	—	Normal
23	C. D.	54	12	Syphilis	+	85	Syst. base	R	0	115	70	0	Large	L.V.P.	7.5	+	2.1	46.8	Normal
24	H. G.	13	12	0	0	82	0	R	+	116	72	0	Normal	Normal	6.5	+	2	81.5	Normal
25	R. J.	28	16	0	0	80	0	R	0	130	90	0	Normal	R.V.P.	5.5	+	3.3	73.9	Normal
26	I. L.	26	8	0	0	100	Syst. base	R	+	116	72	0	Normal	Normal	8	+	1.8	68.7	Normal
27	R. I.	31	9	0	0	105	0	R	0	90	60	0	Normal	Normal	7	+	2.1	59.9	Normal
28	J. B.	68	6	0	0	75	0	R	0	130	90	0	Normal	L.V.P.	6	+	2	56	Normal?
29	H. D.	11	6	0	0	88	0	R	0	98	65	0	Normal	Normal	5	+	1.5	55	Normal
30	Z. S.	30	10	0	0	80	0	R	0	100	72	0	Normal	Normal	6	+	1.8	47	Normal
31	R. M.	29	20	0	0	75	0	R	0	130	80	0	Normal	Normal	—	+	—	—	Normal
32	E. Y.	38	10	0	0	70	0	R	0	114	78	0	Normal	Normal	—	+	—	—	Normal
33	E. S.	23	7	0	±	84	0	R	0	105	70	0	Normal	Normal	7	+	1.8	—	Normal
34	M. F.	29	13	0	0	70	0	R	0	105	72	0	Normal	Normal	6.5	+	1.8	72.6	Normal
35	C. P.	20	6	0	±	85	0	R	0	105	60	0	Normal	Normal	8	+	—	—	Normal
36	B. W.	40	19	Rheum. fever† L & R	+	90	Presys. apex	Irreg.	+	135	75	+	Large	R.V. Extrasystoles	—	+	—	—	Pathologic
37	D. R.	24	6	0	0	95	0	R	0	116	75	0	Normal	Normal	—	+	—	—	Normal
38	L. P.	62	33	0	±	72	0	R	0	140	75	0	Normal	L.V.P.	—	+	1.5	—	Normal
39	J. G.	16	12	Congen. heart dis.	+ Rt.	82	Syst. pul.	R	+	94	68	0	Large	R.V.P.	8.5	+	2.7	35	Pathologic
40	W. Mc.	46	10	0	±	80	0	R	0	115	75	0	Small	Normal	8	+	2.8	80.8	Normal
41	W. S.	38	10	0	0	88	0	R	0	96	66	0	Normal	Normal	—	+	—	—	Normal
42	J. M.	38	5	0	±	92	0	R	0	115	75	0	Small	Normal	7	+	2.8	62.7	Normal
43	C. W.	23	5	0	0	90	0	R	0	110	70	0		Normal	—	+	—	—	Normal
44	E. M.	38	17	Syphilis	±	88	0	R	0	105	65	0	Normal	Normal	6.5	+	2.8	68.1	Normal
45	B. L.	30	8	0	0	78	Syst. apex	R	0	120	75	0	Small	Normal	6	+	2.3	69	Normal
46	C. M.	28	10	0	0	—	0	R	0	120	70	0	Normal	Normal	—	±	—	—	Normal
47	C. W.	23	6	0	0	90	0	R	0	110	70	0	Normal	Normal	—	+	—	—	Normal
48	W. S.	38	10	0	0	88	0	R	0	96	66	0	Normal	Normal	—	+	—	—	Normal
49	C. Y.	46	15	0	0	88	Syst. apex	R	0	110	70	0	Normal	Normal	—	+	—	—	Normal
50	J. H.	49	5	0	0	80	0	R	0	112	72	0	Normal	Normal	6	+	3.2	—	Normal

* Key: R, regular; —, not done; L.V.P., left ventricular preponderance; R.V.P., right ventricular preponderance.

† Rheumatic fever, cardiac disease.

wide under the X-rays, the cyanosis is intense and there is usually a pronounced polycythemia.

Peripheral vascular changes. The most constant circulatory finding in emphysema is an increase in venous pressure. The normal value is about 5 cm. of water (White's method). As emphysema progresses this may reach 20 cm. and above. An elevation of this degree is noted also in cardiac failure, and in obstruction to the vena cava. A comparison is shown in figure 6.

The cause of increased venous pressure in cases of emphysema with normal hearts was investigated and was believed to be due to the increased intrathoracic pressure. Normally, blood is "sucked" into the thorax from the periphery during inspiration. The diaphragm descends and intrathoracic pressure becomes more negative. When emphysema develops, this process becomes interfered with as the pressure within the thorax gradually approaches positive values. The return of blood to the thorax is thereby impeded, and as the blood dams back into the periphery the venous pressure rises.

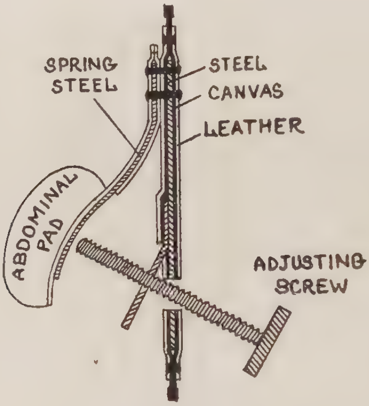


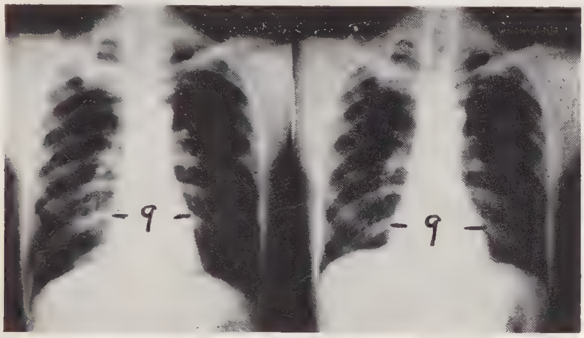
Fig. 5. Drawing of Abdominal Belt; Side View

The canvas belt placed about the lower abdomen laces posteriorly and is held down by thigh straps. The pad is fitted just above the symphysis pubis. The adjusting screw comes through the belt so that pressure can be regulated without unlacing.

This mechanism has been demonstrated experimentally by Kountz and his associates in two series of experiments. In each, dogs with induced emphysema were used and compared with normal controls.

In the first study, simultaneous readings of intrapleural and venous pressures were made during the development of emphysematous lungs. It was noted that after the lungs were distended, the intrapleural pressure rose, to be followed some days later by an increase in venous pressure. The curves, thereafter, maintained a definite relationship.

The finding that increased venous pressure is due to retardation of the flow of blood into the thorax was confirmed by a subsequent study wherein the circulation time was investigated by the method of Hamilton, Kinsman, Moore, and Spurling. A dye tetrabromphenolphthalein was injected into the median basilic vein and the time recorded that elapsed before it could be recovered from the right heart. A distinct delay was found when compared to the rate of normal dogs.



A B

FIG. 6. OBSTRUCTIVE EMPHYSEMA

A, diaphragm is low; *B*, with abdominal belt, diaphragm is pushed up. *9* = level of ninth rib.

The elevation of venous pressure due to a retardation of the flow of blood into the thorax may be responsible for other circulatory phenomena heretofore touched upon. One of these is the development of dependent edema. Although the mechanism by which this takes place is not entirely understood, the experiments of Landis are suggestive. He found that two factors are conducive to edema, namely, increased capillary pressure with a dilatation of the capillary bed, and a lowering of the effective osmotic tension of the blood. The first may be brought about by increased venous pressure, and the second by anoxemia, each of which is a prominent finding in emphysema. No other explanation for the edema has been suggested.

Another effect of the retardation of venous flow into the thorax is the possible effect on the right heart. This was studied experimentally in guinea pigs during anaphylactic chock. The venous pressure, intrathoracic pressure, intra-auricular pressure, and carotid pressure were measured simultaneously, and from these the effective venous pressure in the right auricle was estimated. It was found that the intra-auricular pressure was not increased even when the lungs were greatly distended. This was attributed to the fact that the intrathoracic pressure was so elevated that the venous flow into the right heart was markedly interfered with. This experiment suggests that with lung distention, the right heart instead of overfilling because of obstructed pulmonary circulation, may actually be spared, owing to retardation of the flow of blood entering it. This may explain the fact that in most cases of emphysema, no cardiac lesion is found.

Goëtzl and Kienbock made fluoroscopic observations on the heart during the Valsalva experiment. They noted that when the patient strained against the closed glottis the heart could be seen to decrease in size. We confirmed this observation and found it to be correct. Myer and Middleton studied the venous pressure during the Valsalva experiment and noted that it was elevated. Kountz, Pearson and Koenig observed both the venous and intrapleural pressures during the Valsalva experiment and noted that each was elevated. Both groups of observers believed that with bronchial obstruction the Valsalva experiment is in effect. Consequently, the reduction in the cardiac diameter appears to be due directly to a retardation of venous flow into the thorax.

There is further clinical evidence that this mechanism occurs. Alexander studied circulatory changes during asthmatic paroxysms. He found that after the lungs distended and the venous pressure was elevated, the pulse became small, rapid and thready, and the systolic pressure fell as if there were a lessened volume of blood delivered by the left ventricle.

The arterial blood pressure is usually normal or below normal in cases of obstructive emphysema, in contrast to the senile type where arteriosclerosis and hypertension are common. It is quite possible that the age incidence in these cases may play a part, since the obstructive type is so frequently seen in young asthmatics. However, the rule holds true even in older individuals. This fact is in keeping with the usual absence of heart disease. The infrequency of hypertension, however, suggests the possibility that the cardiodynamics suggested above play a part and that the blood volume in the arterial circulation is actually diminished. This problem is under further investigation.

As a rule, there is no appreciable increase in the number of circulating red blood cells in obstructive emphysema. Hematocrit readings are essentially normal. Cecil Price-Jones, however, found that there is an increase in the size of erythrocytes. He estimated that the mean coefficient of red blood cells in normal individuals is 6.44, whereas in emphysema it may be as high as 7.06. Although this increase is not apparently large, it may explain the absence of elevated total red blood cell counts in most cases. Inasmuch as there is diminished oxygenation of the arterial blood, the finding of a compensatory mechanism is not surprising. In cases associated with sclerosis of the pulmonary artery there is a marked increase in red blood cells, the viscosity is likewise elevated and the solids in the blood may be increased 20 per cent to 40 per cent.

EMPHYSEMA IN ANIMALS

Emphysema is not limited to man alone, but occurs spontaneously and under experimental conditions in certain animals. It appears in its typical form in the horse and cow, and in a modified form in the hog. One case of spontaneous emphysema in a dog has been reported. Its occurrence, however, in the dog, rat, and guinea pig, has been chiefly experimental.

Emphysema was first described in a horse by Floyer. The animal was "wind-broken," a condition brought about by frequent over-exercise without proper training. The outstanding symptom is shortness of breath on light exertion. Autopsy of wind-broken horses characteristically shows typical emphysema.

Another disease which is attributed to emphysema by veterinarians is the so-called "heaves." It is said to be induced in horses by feeding upon musty hay.

Emphysema in the cow resembles that of the horse but is of a lesser degree. The etiological factor is unknown. The condition is believed to occur in old animals, and is less common in domesticated cows than in those raised on a range.

Swine, likewise, are subject to emphysema which is usually induced by the so-called "swine influenza." Lung distention is associated with the pneumonic phase of the disease which closely resembles the bronchopneumonia following influenza in man. Some areas of the lungs may be atelectatic, and others in which there is partial bronchial obstruction are emphysematous. In a group of hogs in which the disease has been studied, it was found that the emphysema persisted for some time after infection. Partial or complete occlusion of the bronchi of swine by parasites is likewise followed by permanent lung distention and loss of elasticity.

Experimental emphysema in laboratory animals dates from the work of Brown-Sequard. He found that electrical stimulation of the vagus nerve and also of the base of the brain near the vagus nuclei caused alveolar distention. Brown-Sequard believed the phenomenon to be due to bronchospasm whereby air becomes trapped in the lungs below the constricted airways. This experiment has been confirmed in our laboratory where an attempt was made to measure alveolar pressure. A capsule was placed upon an exposed lung with celloidin and connected to a water manometer. On vagus stimulation, the pressure in the system increased and the alveoli were seen to dilate. These findings are interpreted as an effect of bronchial constriction.

The emphysema thus produced was acute and no permanent lesion remained. These experiments are in keeping with the well known anaphylactic response in the guinea pig wherein intense acute emphysema occurs. Here, death results because the animal cannot deflate its lungs through the constricted bronchi.

Prinzmetal subjected rats to low barometric pressures and thereby caused great lung inflation. He found that after exposures from one to ten weeks, the alveoli were large with thin and broken walls, characteristic of true emphysema.

Harris and Chillingworth were the first to produce experimental emphysema in dogs by mechanically interfering with respiration. They devised a ball valve which permitted free inspiration but partially obstructed expiration. After insertion into the trachea, emphysema developed within a short time. This work was later confirmed by Kountz, and Alexander, who found that true emphysema with typical changes in the lungs was best brought about by a small degree of obstruction lasting over a period of weeks. The lesions produced were typical. The lungs at autopsy did not collapse readily. They were two to three times larger than normal. The bronchi peripheral to the obstruction were dilated and the alveoli were markedly distended and torn.

Nissen likewise produced a high degree of emphysema in dogs by mechanically obstructing the bronchi by operation. His anatomical description of the lungs is typical of the disease. Seibeck obtained the same result by causing a tracheal stenosis.

Loeb, on the other hand, working with dogs, inserted a tube into the trachea and was unable to cause emphysema. The tube obstructed both inspiration and expiration. He also cauterized the larynx and obtained nothing that either on gross or microscopic examination resembled emphysema of man. His technique was somewhat similar to that of Nissen, although Nissen's points of obstruction were considerably lower, a fact of much significance.

Adams studied the effect on the lungs of bronchial occlusion. He induced complete and permanent closure with 30 per cent silver nitrate. The involved lungs became atelectatic and diminished in size. When a considerable amount of lung tissue had been made functionless by this method, a compensatory emphysema, at times extreme, occurred in the remaining portion of intact lung substance.

Under these conditions the compensatory emphysema, which lasted at times for a year or more, showed anatomical similarities to the disease in man. The bronchi and alveoli were hugely dilated and some tearing of the alveolar walls was noted.

Van Allen's recent experiments indicate that the site of bronchial obstruction is an important factor in determining the extent of the resulting emphysema. His conclusion is based upon the observation that there is collateral circulation of air from one cluster alveoli to another without participation of the bronchi. Hence, if a medium sized bronchus to a portion of a lobe be occluded, or almost so, the air below the point of obstruction does not become trapped in the alveoli since it may seep into contiguous alveoli and be expelled through another bronchus. If, however, a small bronchus, supplying a terminal segment where no collateral circulation is possible, be partially obstructed, the forces of expiration are not sufficient to expel air and alveolar distention results. Van Allen pointed out that in man each lung is separate so that stenosis of a main bronchus or of the trachea will have the same effect. In dogs, one lobe is occasionally attached to another, in which case obstruction to egress of air will not be followed by emphysema, since trapped air will find its way into the contiguous lobe. In bronchial asthma in man, emphysema results inasmuch as many small bronchi (those 4 mm. to 6 mm. in diameter) are simultaneously contracted.

Bullara studied the part that nasal occlusion plays in the production of emphysema by obstructing the nares of dogs. In time, some emphysema in the lungs occurred.

PATHOGENESIS

By separating cases of emphysema into the obstructive and postural types, the consideration of the etiology of the disease becomes simplified.

Mechanical theories. Laennec believed that emphysema could be explained entirely by mechanical forces. He contended that all cases begin as a catarrh of the bronchi. Mucus is thus produced which causes partial bronchial obstruction to both intake and discharge of air from the lungs. A greater respiratory effort results thereby, and since the forces of expiration are weaker than those of inspiration, the expulsion of air is impaired. As a result, air gradually accumulates in the alveoli, whose walls become distended and even rupture, and emphysema thus becomes established. This theory of Laennec was greatly misinterpreted at first, and soon was vigorously attacked by Louis, Andral, Piedaguel and others on various grounds. Emphysema was considered by some, at that time, as merely an hypertrophy of the lungs, by others an atrophy (of the alveolar walls), whereas others denied the existence of vesicular emphysema and contended that all cases were interlobular in origin. Some years later, however, Laennec's theory of bronchial obstruction as the underlying cause of the disease began to receive support, principally on clinical grounds, and is now accepted as an outstanding probability.

Brown-Sequard was the first to verify this contention by animal experimentation. Since then various experimental methods of causing partial obstruction to expiration, which have been described, have resulted almost uniformly in distention of the lungs.

These observations have clinical support, particularly in the fact that emphysema is almost constantly associated with cases of long-standing bronchial asthma and with chronic bronchitis. There is dispute as to whether occupational emphysema occurs. Several of the older authors have stated that glass blowers and musicians who play wind instruments are liable to develop the disease. Lommel found the vital capacity to approximate normal in a series of glass blowers under 40 years of age. Becker studied the ratio between the vital capacity and the midpoint of breathing in twenty-two players of wind instruments, and in nineteen the values were at the lower limits of normal. In each of these groups the absence of emphysema was explained by the moderate degree of obstruction and by its intermittency.

Opposed to the contention that emphysema is caused by impairment to expiration, an "inspiratory theory" was championed by Williams, Gairdner, and Elliotson, who maintained that alveolar pressure cannot be built up by bronchial obstruction to a degree that will deform the chest and rupture alveolar walls. Only the forces of inspiration can bring about these profound results. The inspiratory theory was based upon the assumption that a pre-existing disease of the lungs such as tuberculosis, extensive bronchitis and other conditions causing fibrosis diminishes the amount of functioning lung tissue. Exertion and other unusual demands on respiration thereafter overstretch the remaining alveoli as they follow the chest wall and diaphragm to their inspiratory positions. The emphysema that results is essentially a compensatory process.

It came to be believed that pre-existing pulmonary disease is not essential to the production of inspiratory emphysema, and that the condition may be brought about merely by increased respiratory effort by apparently normal lungs. Podkaminsky examined the lungs of a group of individuals who had been subjected to hard physical labor. He found that the size of the organs, air content, and volume were proportionate to the work done. Likewise, Engelhard noted that emphysema had developed in individuals who were overworked, and that the process was a functional hypertrophy of the lung. Durig and Hasselbach found that after strenuous exercise such as mountain climbing, an acute emphysema occurred and lasted several days after the exertion. Hurtado measured the thorax of individuals living at very high altitudes. He observed an apparent emphysema in that the chest assumed an inspiratory position with hyperresonant lungs. The experiments of Prinzmetal, who produced typical lesions of emphysema by exposing rats to low barometric pressures have been described. Campbell obtained similar results which indicate that prolonged or intense inspiratory effort may induce emphysematous lungs.

A number of observers, on the other hand, maintained that no single inspiratory or expiratory factor causes emphysema. These authors whose work was based largely upon clinical and anatomical studies, and to a lesser extent on direct experiment, believed that both expiration and inspiration each play a part in the production of the disease.

Another mechanical theory of the cause of emphysema has received considerable attention. This was advanced by Freund, who, after a study of a large group of cases, concluded that emphysema results from faulty position of the chest rather than that it is primarily a disease of the lung. He believed the primary lesion to be an ossification of the costal cartilages with a subsequent bony fixation of the chest anteriorly. The chest would thereby assume an inspiratory position, and the lungs remain distended.

Loeschke in his notable monograph on emphysema concluded that the disease may be brought about by various factors, among which he considered spinal deformity, particularly kyphosis, one of the most important. He has shown that angulation, particularly of the lower dorsal vertebrae may bring about an enormous enlargement of the thoracic cage. This, in turn, causes an extreme stretching of the lungs, which becomes permanent. The emphysema may be general or, if the chest is asymmetrical, limited to the portion of the thoracic space that is enlarged. These factors will be discussed further under "Postural Emphysema."

Degenerative theories. Under this broad heading are included theories which deal primarily with degeneration and destruction of various elements of lung tissue. One that has received much attention deals with a diminution of elastic tissue. The failure of emphysematous lungs to collapse when the chest is opened, as well as the appearance of atrophied stroma, have suggested that the elastic tissue element is at fault. Pathological studies by Eppinger and Letulle have supported this impression in that on examination elastic tissue was found to be diminished. On the other hand, Klaesi and Tendeloo have been unable to confirm these observations.

Rainey and Isaacsohn were the first to announce that emphysema is due primarily to disease of the vascular structures in the lung, particularly the smaller branches of the pulmonary artery. They supported this theory by the facts that emphysematous lungs contain less blood than normal lungs, and that they observed fatty degeneration with thrombi in the lumina of the vessels themselves. They cited the observation that elderly people with emphysema often have arteriosclerosis also. Curschmann believed that emphysema is arteriosclerosis of the lung much as Bright's disease is arteriosclerosis of the kidney. Delafield made the same assertion.

Villemin and Biermer found evidences of nutritional disturbances in emphysematous lungs. They observed fatty degeneration of the alveolar epithelium which they attributed to toxic factors, and believed this condition to be a forerunner to elastic tissue degeneration. With these lesions, even the ordinary depth of breathing would cause dilatation and destruction of the alveolar walls.

Minkowsky and West noted the frequent incidence of pulmonary infection that is followed by emphysema, pointing out that the infection may cause sufficient capillary damage to interfere with the area of lung involved. Nutritional changes follow with consequent loss of elastic tissue. This point of view is supported by the studies of Grawitz, who found that following leucocytic infiltration about the alveolar walls as the result of infection, there is destruction of lung parenchyma with loss of elastic elements. These degenerative changes progress to the rupture of alveoli. Pronounced emphysema following the bronchopneumonia of influenza has been described by Torrey and Grosch. The lesion in these cases surrounds the small bronchi. Emphysema associated occasionally with infection and atrophy of the bronchial walls has been described on page 259. It is noteworthy that in cases of this type which we have had occasion to study, there was no evidence of alveolar involvement in the infectious process. The lesion was uniformly in or about the bronchial walls. The contention of Grawitz could not be confirmed.

Many authorities assert that emphysema is an atrophy of the lung, but there is much discussion as to whether this atrophy is the result of degenerative changes which lead to weakening of the alveolar walls or whether it is secondary to stretching.

There are numerous theories dealing with degeneration of intercostal muscles, disturbed metabolism, trophic changes from interference with the nerve supply to the lungs, vagotonic influences, and other etiological factors, none of which have received support.

From the data presented, it appears that emphysema may arise from many causes. As pointed out by Loeschke, however, it becomes necessary to distinguish between conditions which result in permanently enlarged and ruptured alveoli and those of mere lung distention which is not chronic emphysema in the clinical sense.

There is every indication that by far the largest number of cases of emphysema, with the exception of the postural type, follow bronchial obstruction. The most frequent underlying diseases are asthma and chronic bronchitis. As was discussed elsewhere, some patients with typical obstructive emphysema, but with no history of a bronchial lesion, were discovered to have an undetected bronchospasm. The fact that in experimental animals, prolonged interference with expiration uniformly leads to lung distention, as well as the observations of Alexander, Luten and Kountz that in a series of 50 patients with longstanding asthma, all but one had pronounced emphysema, tend to verify this contention. It seems unnecessary to assume an inherent defect in the lung, a supposition which has been insisted upon by some authors.

That there are occasional cases of pronounced chronic emphysema without any demonstrable evidence of bronchial obstruction is unquestionable. One group of these is caused by deformities of the spine, particularly kyphosis. Another group includes the familial cases which may develop in childhood, and also the infantile type. Here, the etiology has been attributed to a congenital defect in the elastic tissue element of the lung. The advanced stage of emphysema associated with pulmonary arteriosclerosis (Ayerza's disease) is thought to be due primarily to interference with lung nutrition. The widespread involvement of the pulmonary artery down to its finest branches which enter into the structure of the alveolar walls points to this supposition.

The apparent emphysema caused by excessive inspiratory effort, which has been an outstanding theory of the disease, deserves attention. There is no question that overwork, especially in those unaccustomed to it, may cause lung inflation. In man, this leads to acute emphysema, which, however, disappears shortly. In wind-broken horses, on the other hand, typical lesions are found. The observations of Podkaminsky and of Erhart that individuals subjected to hard labor have lungs larger than normal may be looked upon as a work hypertrophy, rather than true emphysema. Certainly, clinical experience does not support the contention that prolonged physical exercise causes permanent alveolar distention, for the large incidence of the laboring class in hospital wards is out of all proportion to the number of cases of the disease. The apparent emphysema of those living at high altitudes is doubtless due to lung inflation from reduced atmospheric pressure. Hurtado looks upon this as a physiological process. It is questionable if true emphysema exists under these circumstances, for clinically, respiratory function is adequate. Moreover, at autopsy patches of atelectasis and of widened alveoli, pronounced engorgement of the capillaries, and some loss of elasticity were the essential lesions. This is not a picture of obstructive emphysema.

In cases associated with infectious lesions of the bronchi whose walls are atrophied, the mechanism by which emphysema is produced remains in doubt. The fact that the parenchyma of the lungs does not appear to be involved points to the bronchi as the primary seat of the lesion. Anatomical descriptions of emphysematous lungs deal only with cases far advanced, so that the earlier processes remain unknown. It would seem possible that since the lesion primarily surrounds the bronchi and gradually encroaches upon the walls toward the lumen, a partial bronchial obstruction similar to the peribronchial lesion in the pneumonia following influenza may occur and be sufficient to cause emphysema. Destruction of the bronchi may continue long after emphysema has been established. Until earlier stages of the process have been observed, the true sequence of events must remain in doubt.

It appears, therefore, that, excluding the postural type, by far the greatest number of cases of pulmonary emphysema as defined by permanent alveolar distention and rupture are due to partial bronchial obstruction. A few can be attributed to other causes. In those instances where inspiratory factors lead to lung inflation, emphysema is simulated, but it is very doubtful whether characteristic lesions are produced.

SYMPTOMS, PHYSICAL SIGNS, COMPLICATIONS AND DIFFERENTIAL DIAGNOSIS

Symptoms. The onset of chronic obstructive emphysema is slow and insidious, but the acute form may develop rapidly. An attack of bronchitis, whooping cough, or asthma, or partial compression of the trachea or a large bronchus, may result in distended lungs. Under these circumstances, emphysema usually disappears after the cause has been removed, but if attacks be repeated, and occasionally after a single attack of influenzal pneumonia, permanent emphysema may follow. Lichtenheim observed emphysema to persist in children following diphtheria with laryngeal obstruction of six days' duration. In other words, partial bronchial obstruction is followed by temporary trapping of air in the alveoli, whereas prolonged or repeated obstruction results in a loss of elasticity and rupture of the alveolar walls.

Dyspnea is by far the most important and prominent symptom of chronic emphysema. The patient gradually appreciates an increasing shortness of breath after unusual exertion, although he is entirely comfortable while at rest. As time goes on the dyspnea in most cases tends to become more and more associated with the cough of the underlying bronchitis or asthma in that it grows increasingly difficult for the patient to regain his breath during and after an attack. The bronchi become irritable, so that dust, fumes, or sudden cold air, may set up a spasm of coughing or there may be paroxysms of dyspnea with wheezing, even in individuals who previously never had had asthma. These attacks occur especially in the early morning hours. In time, the lungs become so distended that the patient is entirely incapacitated for work. When this takes place, the vital capacity is usually below 2000 cc. In fatal cases, bronchopneumonia appears, as a rule, but the patient may die of emphysema itself. In such instances there is increasing orthopnea which becomes extreme and cyanosis is intense.

Either sudden collapse with death occurs or there may be hours or days of coma. These terminal events are not common, for emphysematous individuals carry their affliction for many years and usually die of intercurrent disease. Moreover, the process may progress so slowly that it appears to be arrested. It is surprising how much work some of these patients can accomplish in view of their low vital capacity. This has been explained on the grounds of increased tolerance for CO_2. Sooner or later, however, complete disability usually occurs.

As emphysema progresses there is frequently a deterioration of general health. Anorexia is common and has been attributed to various causes such as the swallowing of bronchial secretion, the mild fever of the associated bronchitis, and to constipation from lack of exercise. Flatulence is a frequent symptom. There is usually loss of weight. The skeletal musculature becomes flabby, and the lower abdomen protrudes when the patient stands. Headache and drowsiness are frequent.

Occasionally, pain in the epigastrium is an early symptom. This was studied by de Buen, who noted it in over one-half of a series of cases. The point of pain is located about the xyphoid cartilage, and pressure over the epigastrium increases the distress.

Physical signs. On inspection, patients with chronic obstructive emphysema present a familiar picture. In most cases undernourishment is apparent. The thorax is rounded or barrel-shaped, and the anteroposterior diameter increased. A costometric tracing may show that it exceeds the transverse. The clavicles are prominent, the supraclavicular and infraclavicular fossae deeper than usual, and the shoulders are rounded, causing the scapulae to flare. The thorax is partially fixed in the inspiratory position, and the sternoclavicular and trapezius muscles are prominent and hypertrophied. The first and second pieces of the sternum sometimes form a prominent angle (of Ludovici), the bone being convex from above downward. The intercostal spaces are wide, the subcostal angle is increased while the flare of the lower ribs gives the abdomen a hollow appearance.

Cyanosis varies from bluish lips to a dusky flush of the cheeks. Osler remarked that there is hardly any other condition in which a patient may go about and even walk to a hospital with cyanosis of so startling intensity. The finger nails not only are blue, but in most cases where anoxemia is pronounced, Hippocratic finger tips are found. Hoover pointed out that ballottement of the nail against its bed reveals a resistance similar to that which a soft shell crawfish gives to the compressing finger. He considers this an early sign of the edema that accompanies anoxemia. Such edema which is soft and pitting occasionally occurs in the lower extremities. The veins of the neck and upper extremities are distended. Frequently, dilated venules are present over the lower thoracic region at the level of the attachment of the diaphragm. The heart impulse is rarely visible. Marked cardiac pulsation however appears in the epigastrium because of the low diaphragm.

The type of breathing of obstructive emphysema is notable. As the lungs inflate, the diaphragm descends until it takes the position of extreme contraction and can no longer function. As this occurs, normal abdominal breathing diminishes and finally ceases entirely. Lung expansion thereafter is dependent upon the excursion of the thorax, but since the chest at this stage of the disease is already distended (barrel chest), respiration is greatly restricted. Actually, chest expansion may not exceed one-half inch. The accessory muscles come into play, especially if the patient exerts himself. Not only is costal breathing apparent on inspection, but fluoroscopic examination reveals the flat diaphragm which may be fixed. In extreme cases, paradoxical movement is observed in that the diaphragm actually elevates during inspiration as it is drawn upwards by the reduced intrathoracic pressure which is created while the chest expands. Under this circumstance, an actual recession of the upper abdomen is seen as the breath is drawn in.

There has been controversy concerning the factors entering into emphysematous breathing. It is commonly supposed that since the elasticity of the lungs is impaired, the intercostal and abdominal muscles contract to compress the chest and thus assist in squeezing air out of the lungs. Hoover, particularly, studied this problem, and found that one may determine whether active expiration is being employed merely by palpation of the abdominal wall. If this contracts as the patient breathes out, it is dependable proof that the intercostal muscles are compressing the chest. Although this observation of Hoover is probably correct, it is doubtful whether contraction of skeletal muscles is the sole mechanism by which air is forced out of the lungs. Active expiration is an exhausting process and only in periods of extreme dyspnea such as during an asthmatic attack is it resorted to. Likewise, in animals this method of breathing is employed only when air hunger is intense. The possible participation of the bronchial musculature in assisting expiration should be considered. Although this is by no means established, the work of Miller, Heinbecker, Hudson and others is strongly suggestive. In emphysema the dyspnea is often improved by epinephrin, which suggests that the bronchi may play an active part in breathing.

On palpation of the chest, vocal fremitus is diminished. Except in the rather rare cases of marked cardiac hypertrophy, the apex beat when felt is nearer the sternum than normally.

Percussion, if properly interpreted, is of considerable value in diagnosis. The note is variously described by observers who fail to differentiate between a lung that has become acutely distended and one that is enlarged as a result of bronchial obstruction with atrophic alveolar walls and enlarged lobules that are incapable of contraction. Such terms as "hyperresonant," "loud and deep," "tympanitic," "box tone," and "short and high pitched" have been used. Hoover pointed out that all these descriptions are correct for different cases. In acute emphysema where the lung has not lost its capacity for distention and contraction the note has a fairly long duration and may have a low pitch. If the lungs are chronically distended, the note is shortened and the pitch elevated. Hoover further called attention to the fact that in chronic emphysema there is an increased resistance to the percussing finger which varies with the degree of increased intrapleural pressure. The resonance is over the entire chest, masking cardiac dullness, unless very light percussion is used. It extends abnormally low, for the lungs displace the diaphragm and abdominal viscera downward and partly obliterate liver dullness.

On auscultation, inspiration is increased in intensity and of about normal duration. Expiration is prolonged and distant. The pitch of both inspiration and expiration elevates with the degree of obstruction present. In most cases a few musical râles are audible, particularly during expiration. During an acute exacerbation of bronchitis moist râles appear. The whispered and spoken voice are distant.

Occasionally cavernous breathing is heard. According to Fleckseder, this occurs only when the cavity is in communication with a bronchus, the respiratory movements are large, the cavity fixed and surrounded by fairly dense sound conducting tissue. The heart is heard with difficulty.

In obstructive emphysema, the thoracic portion of the spine assumes a continuous curve as it bows posteriorly. It has been suggested that this results from an unconscious posture which the patient assumes in an effort to compress his lungs. By standing erect, the ribs separate; by stooping they approximate. This fact will be elaborated in the section on "Postural Emphysema."

Fluoroscopy is an important aid in diagnosis and confirms many physical signs. The lung fields are unusually well illuminated, since they contain more air than normal. The intercostal spaces appear widened. The diaphragm is low and its arches flattened, with little or no excursion. The heart, which is pulled downward by its diaphragmatic attachment, assumes a tubular or spindle shape, and is displaced toward the median line. The pulmonary vessels are frequently prominent. Herms considered this sign presumptive evidence that emphysema is related to some change in the pulmonary artery. A more probable explanation of the increased vascular shadows is that pulmonary markings are faint in the brightly illuminated lung fields, and the heart and large blood vessels thereby become conspicuous.

Complications. Pneumothorax may occur from rupture of emphysematous blebs. Siems reported three cases and believed that blebs anywhere on the surface of the lungs can be torn. Fischer, on the other hand, contended that rupture occurs only at the apices where the intrapleural pressure is greatest. Pneumothorax is a serious complication in emphysema in that breathing is further handicapped. In one patient of our series, the vital capacity was reduced below 900 cc.

Frank hemoptysis is not frequent. It is attributed to rupture of vessels within weakened lung walls or in bronchial cavities. Recurring hemorrhages are usually slight, but have been reported as severe and fatal. Blood streaked sputum accompanying bronchitis is not unusual.

Interstitial emphysema is associated particularly with acute emphysema, especially that caused by bronchopneumonia following influenza. The air beneath the pleura may remain confined within the chest or it may escape along the vessels of the neck and retro-peritoneal area, and finally appear in the subcutaneous tissues.

Occasionally, when cardiac failure occurs in patients with emphysema the usual signs of decompensation become accentuated and cyanosis is the most prominent. Increasing dyspnea, even with the patient at rest is also notable. Dependent edema involving the legs, sacral region and scrotum is marked. As pointed out before, however, marked edema may result from increased intrathoracic pressure resulting from emphysema alone. Râles appear early and their disappearance is usually the first sign of improvement. The blood pressure is characteristically low. If the heart is much enlarged a mitral systolic murmur appears. Pulsation of the liver is associated with right heart failure.

Early cardiac involvement in obstructive emphysema is difficult to detect. The most reliable method is X-ray examination. The usual picture is enlargement of the cardiac shadow both to the right and left in contrast to the characteristic elongated heart outline. With right heart failure, as occurs in emphysema associated with Ayerza's disease, although right-sided enlargement is present, the prominent shadow of the left auricle seen in mitral stenosis does not appear. Pulsation of the lung fields (Ulrich's sign) may be seen. It is noteworthy that in emphysema the heart frequently appears on X-rays to be displaced to the right, a finding that is not confirmed at autopsy.

Bronchiectasis, pulmonary tuberculosis, and various forms of pneumonokoniosis are associated conditions rather than complications.

Differential diagnosis. The diagnosis of chronic obstructive emphysema is not difficult, although it is surprising how frequently the condition is mistaken. Postural emphysema more than any other condition simulates the obstructive type.

Acute emphysema is associated with or immediately follows its etiological agent. It may appear after unusual exercise, ascent to high altitudes, during or just after an asthmatic paroxysm, or with various bronchial afflictions which cause partial obstruction. Its presence is detected by physical signs, especially percussion as noted above. It rarely approximates the intensity of advanced obstructive emphysema excepting during a severe asthmatic attack, and occasionally in influenzal pneumonia. Chronic signs, as Hippocratic fingers and dilated thoracic venules, are absent. Acute emphysema is of short duration and usually disappears completely.

Compensatory emphysema is localized to the unaffected portion of the lung. Its title implies a coexisting pulmonary lesion. The lungs have a remarkable power of expansion, and in cases where a lower lobe is atelectatic and contracted, the middle lobe may fill the lower chest on that side. When an entire lung has lost its function, the opposite side may distend to such an extent that the thorax over it becomes much enlarged. If the process is pronounced or of long standing, loss of elasticity and rupture of the alveoli occur and true emphysema becomes established. As a rule however, compensatory emphysema has little clinical importance and is easily recognized.

In advanced visceroptosis, the diaphragm may be pulled down by the weight of the liver, if the abdominal wall is flaccid. With the patient erect, the lungs are stretched vertically and the bases are low. The thin chest wall accentuates the percussion note. Such cases may readily be mistaken for obstructive emphysema at first glance, since abdominal breathing is limited or absent. Moreover, in individuals with hyposthenic habitus who have emphysema, the barrel chest is not always conspicuous. A differential diagnosis can be made with the patient recumbent when the diaphragm is pushed up again and permitted to descend. Abdominal breathing is then resumed. In obstructive emphysema posture has no effect on diaphragmatic movement which is abolished by the distended lungs.

TREATMENT

The fact that emphysema is a consequence of prolonged bronchial obstruction has served to detract attention from it. The symptoms of the underlying asthma or bronchitis appear more immediate, and as a rule, emphysema is well advanced before its significance is appreciated. The institution of treatment during the early stages of the process would probably accomplish much. The most ready way of estimating the degree of emphysema is to determine by X-ray examination the position and excursion of the diaphragm, and the clearing of the heart shadow.

In the early stages of the disease, prophylactic measures such as an anti-allergic régime and treatment of bronchitis, directed toward protecting the patient from factors which constrict the bronchi, are indicated. Exertion should not be extended to the point where the lungs become stretched. The importance of this has been pointed out by Schesinger. The maintenance of good general health by diet, cold baths, and other hygienic measures serves to counteract the general deterioration occurring with advanced emphysema. A good deal of attention, especially in Germany, has been paid to posture and to breathing exercises with satisfactory results. The patient is taught to stand erect to prevent bowing of the spine. A well applied back brace is helpful. On inspiration the abdomen is drawn in forcibly to help elevate the diaphragm. This will assist in expelling air and at the same time improve excursion during inspiration. An abdominal belt, described below, may protect the diaphragm and help expulsion of secretions from the bases of the lungs.

Once the disease is in an advanced stage, the problem of therapy becomes much more difficult. Frequently associated asthmatic paroxysms wherein the lungs become still more distended mask the dyspnea of the underlying emphysema. Relief of attacks becomes the first consideration.

A clearer picture of the effect of underlying lesions on severe emphysema is often observed in cases with an active bronchitis. If the patient be put to bed, given steam inhalations, cough mixtures and sedatives as indicated, he will usually show marked improvement in breathing. This is accompanied by an elevation of the vital capacity, often to a considerable degree. In some clinics, digitalis is given such patients on the assumption that the dyspnea is cardiac in origin, a theory which seems supported by subsequent relief. The same degree of improvement, however, may usually be secured without digitalis, excepting in those few instances where the heart is seriously involved.

The good results obtained by relieving the acute phases of chronic emphysema cannot be explained by the assumption that periodic coughing and secretion in the bronchi alone can affect alveolar distention. Some other factor appears to be involved. Jagic and Spengler, from clinical studies, maintained that in bronchitis some spasm of the bronchi occurs and that acute cases may be improved by adrenalin.

Bronchospasm would readily account for the pronounced effect of an acute bronchitis upon emphysema. This possibility is supported by clinical observations stated in a previous section, wherein adrenalin given to cases of emphysema caused a marked rise in vital capacity.

Ephedrine in doses of 50 mgm. given at spaced intervals of every four or six hours has proven to be a valuable drug. This is true in patients at bed rest during the acute phase, as well as in ambulatory cases. While taking it, patients appear to be capable of an increased amount of exertion. When the dyspnea of emphysema is acute, small doses of epinephrine (0.2 cc. to 0.4 cc.) are immediately effective. Various mixtures designed to relieve cough and at the same time to relax bronchospasm have been tried. One containing tincture of belladonna 0.3 cc., potassium iodide 0.3 gram, codeine sulphate 0.015 gram to 4 cc. of elixir lacto pep. has been satisfactory.

A patient with emphysema and bronchitis is usually improved during warm weather, and on the whole is better off when winter climate can be escaped. If this is not possible, he should be instructed to dress warmly with woolens next to the skin. Cold baths in the morning, beginning in the summer and continued throughout the winter months, are often beneficial.

The treatment of dyspnea occasioned by the distended lungs has been directed entirely toward the correction of the barrel chest and the depressed diaphragm. The earliest attempt was the Freund operation wherein costal cartilages were severed. The rationale of this procedure was based on the assumption that the distended thorax is the primary lesion and that the lungs enlarge by following the chest to its abnormal position. The Freund operation had considerable vogue for a time, but eventually was discarded, although a number of successful cases have been reported. Seidel recommended a resection of the second to the fourth costal cartilage in a two stage operation.

Various mechanical devices to facilitate breathing have been advocated from time to time. These included pneumatic cabinets, in one of which compressed air encompassed the body during expiration so that a large inspiratory excursion would be possible. Another (the Waldenburg apparatus) could be so regulated that the patient inspired outer air but exhaled into rarified air, a procedure designed to reduce lung volume. The Bruns apparatus was of a similar nature but cheaper and more easily handled. The Rossbach breathing chair and the Boghean breathing machine were mechanical instruments in which the patient was placed and the abdomen compressed during expiration. The Hofbauer apparatus carried out this process more simply. It consisted of a bag placed around the abdomen and attached to a tank of compressed air. A timing device permitted the bag to fill with air during expiration. As a result, abdominal compression was transmitted to the thorax and air was thus expelled from the lungs. Breathing exercises and manual compression of the thorax for a period each day by a masseur were measures also recommended to overcome the mechanical handicaps to breathing in emphysematous individuals. Most of these procedures were either too cumbersome to be of general use or not sufficiently effective to be generally adopted.

TABLE 4

Effect of an abdominal belt on the vital capacity of the lungs

PATIENT NUMBER	ETIOLOGY OF EMPHYSEMA	VITAL CAPACITY		DECREASE	REMARKS
		Before belt	With belt		
		cc.	*cc.*	*per cent*	
1	Bronchitis	1,800	3,200	77	Improved
2	Bronchitis	2,500	3,300	32	Improved
3	Asthma	1,400	2,200	57	Unable to wear belt
4	Bronchitis	2,200	3,300	50	Improved
5	Influenza	2,500	3,500	40	Improved
6	Asthma	1,700	2,500	47	Would not wear belt
7	Asthma	2,500	3,200	28	Improved?*
8	Bronchitis	3,000	3,700	23	Improved?*
9	Pulmonary sclerosis	1,000	1,400	40	No improvement
10	Bronchitis	2,900	3,600	24	Improved
11	Bronchitis	2,400	3,300	37	Improved
12	Asthma	2,250	3,700	64	Improved
13	Bronchitis	1,500	2,500	66	Improved
14	Indeterminate	2,250	2,800	24	Improved
15	Influenza	3,500	4,200	20	Improved

* Failed to return.

In 1933, Alexander and Kountz constructed a simple abdominal belt which gave satisfactory results in a small series of cases in which it was tried. With this, pressure exerted just above the symphysis pubis is transmitted upwards so that the diaphragm is pushed toward its expiratory position. Anteriorly, a steel band carries a pad which is directed backward toward the promontory of the sacrum. The pressure exerted by the pad is regulated to suit the patient's comfort by a screw which can be adjusted.

The effect of abdominal pressure upon the position of the diaphragm is shown in figure 6.

A series of fifteen patients with marked obstructive emphysema was studied from the standpoint of increased vital capacity and subjective improvement after the belt had been worn for one to six months (table 4). The average vital capacity without the belt was approximately 2300 cc. With it, it was approximately 3100 cc., an increase of 34.8 per cent.

The effect of raising the diaphragm on intrathoracic pressure was studied by records of intrapleural pressures. Before application of the belt, characteristic elevated pressures were observed. After application, the intrapleural pressure became decidedly more negative. This result was apparently due to the activity of the diaphragm, inasmuch as intrapleural pressure becomes more negative as the activity of this muscle of respiration increases.

POSTURAL (SENILE) EMPHYSEMA

The foregoing sections have dealt with obstructive emphysema, which presents a definite clinical entity. The usual history of partial bronchial obstruction, the voluminous lungs and barrel chest, the low position of the diaphragms with costal breathing, the reduced vital capacity and the cyanosis identify the disease. Another type of emphysema has long been recognized, namely the "senile" or "arteriosclerotic," which is commonly seen during or past middle age and characterized also by a barrel chest and hyperresonant lungs. Although this form of emphysema has been distinguished clinically from the obstructive type, there has been a persistent tendency to consider one a variation of the other, and frequently to ascribe a common cause to both. This treatment of the subject has led to much confusion and is largely responsible for the many theories of the etiology of the disease. For instance, the presence of emphysema in elderly individuals has led to the assumption that degenerative processes underlie changes in the lungs, and this theory has been extended to account for all cases of emphysema. Conversely, since bronchitis is frequently associated with obstructive emphysema, and is common in old age, senile emphysema has been presumed to be due likewise to bronchitis. In recent monographs, the problem has been met by the assertion that emphysema may result from many unrelated processes. This attitude has confused rather than clarified the problem.

In 1932 Alexander and Kountz presented evidence which demonstrated that the condition known as "senile emphysema" is an entity whose etiology and clinical picture are entirely distinct. In all cases, they found a stiffness of the thoracic spine. On bending forward or backward, the axis of rotation was through the lumbar vertebrae, or if these were likewise involved, motion occurred only at the hips. The influence of a stiff thoracic spine on the development of emphysema at first was not apparent. In some cases, however, there was not only stiffness but the upper portion of the spine appeared straightened in that the normal backward bowing was absent. It was then found that this straightening of the thoracic column occurred in all early cases, but was not always apparent on inspection. The effect could be seen, however, on x-ray films taken in the lateral position, and at autopsy.

The mechanism by which the thoracic spine assumes a more vertical position, and the influence of this deformity on the thoracic cage, was studied by means of models. It was noted that as the spine straightens, the vertebral bodies separate. Inasmuch as the ribs are attached posteriorly to the vertebrae, straightening of the dorsal spine causes a separation of the ribs and a barrel chest is thus produced. This effect could be demonstrated by stripping the muscles from the thoracic cage of a normal cadaver and setting the base of the spine and pelvic bones in plaster of Paris. When the spine was straightened so that the thoracic curve was lost, the sternum elevated and the horizontal diameters increased.

It is apparent, then, that a barrel chest, which is a constant sequel of obstructive emphysema and resulting from prolonged intrathoracic pressure, may also be produced by a deformity of the spine, regardless of any pulmonary lesion. In such instances, the lungs follow the chest wall and are hyperresonant. As will be shown, however, unless

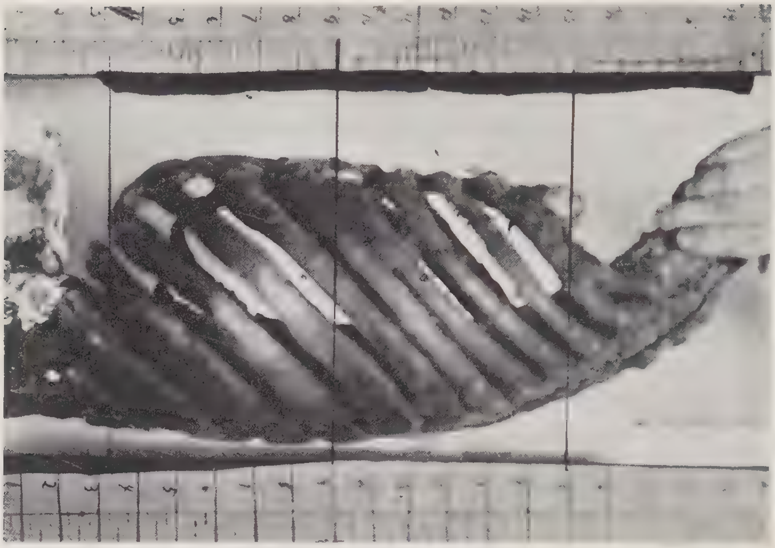


FIG. 7. THORACIC CAGE OF A NORMAL CADAVER FROM WHICH ALL MUSCLE HAS BEEN REMOVED

The upper edge of the sternum stands at 15 inches on the scale

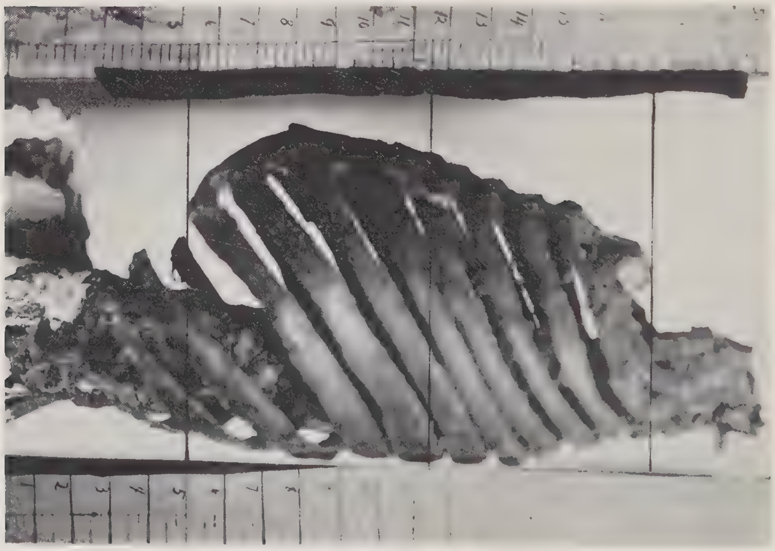


FIG. 8. THE SAME MODEL AS FIGURE 7

The thoracic spine has been straightened. The upper edge of the sternum stands at 17 inches. The depth of the chest from the spine to the sternum has been increased $1\frac{1}{4}$ inches.

the barrel chest be extreme, there is no great enlargement or loss of elasticity of the lungs, which promptly collapse when the chest is opened. They merely shift their position. The term "senile emphysema" as applied to cases resulting from spinal deformity is misleading, for, although straightening of the upper vertebral column occurs usually after middle life, a barrel chest may result from kyphosis of the spine found in younger individuals. The condition is therefore referred to as "postural emphysema."

Alexander and Kountz were not the first to contend that emphysema may be caused by abnormalities of the thoracic cage. Freund, Meyer, Hofbauer, Loeschke, and others have emphasized the fact that kyphosis may result not only in a barrel chest but the thoracic deformity may be so great that the lungs are stretched and the alveoli torn. A true emphysema may be thus produced. The mechanism of the production of a barrel chest from kyphosis of the dorsal spine depends primarily upon the fact that as the thoracic column collapses it moves away from the sternum, which is fixed anteriorly by the clavicles and ribs. The antero-posterior diameter of the thorax is thereby increased. A second factor involves the sinking downward and forward of the upper segment of the collapsed spine. To accommodate for this altered position, the ribs spring outward and the horizontal diameters of the thorax increase. A barrel chest resulting from this process may be visualized in a model in which the fourth dorsal vertebra has been partially cut away and the upper spine pushed forward and downward.

Loeschke has shown schematically that the lower the kyphosis, the more extreme the barrel chest. He and others also have demonstrated that not only may great stretching and rupture of the lungs result, but that this lesion is localized particularly to the horizontal plane of the kyphosis. This observation may be confirmed both clinically and pathologically in individuals with Pott's disease of the spine.

Other investigators have attributed the production of emphysema to abnormalities of the thoracic cage. The work of Freund is well known and has been described. If Freund's theory were correct, one would expect to find the lesion he described in all cases of postural emphysema, whereas it is by no means constant.

Creuyx believed that he could demonstrate degenerative changes in the intercostal muscles by studying response to electrical stimuli.

He stated that this permits an abnormal position of the thoracic cage which, because of the dynamics involved, would assume the inspiratory position. Creuyx's experiments were repeated, and although there is some evidence to suggest a weakness of the muscles of respiration in emphysema, we believe with Clement that such muscle degeneration is a result of bony deformity and thus a secondary phenomenon rather than a primary disease of the muscles or nerves which innervate them.

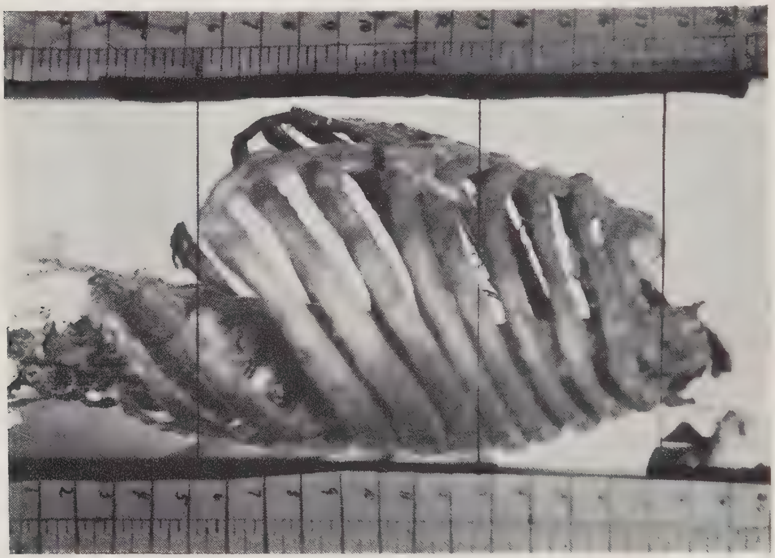


Fig. 9. The Same Model as Figures 7 and 8

A portion of the fourth dorsal vertebra has been removed. Pressure is directed downward and forward. The sternum is raised to $17\frac{1}{4}$ inches and the depth of the chest has been increased $3\frac{1}{4}$ inches.

Hofbauer suggested that the fixed chest of emphysema is due to overaction of the inspiratory muscles over those of expiration. The chest would thereby distend and finally mobilize. We found no experimental or clinical evidence to support this theory.

By far the greatest number of cases of postural emphysema are those in which the process begins as a stiffness and later a straightening of the thoracic spine, the commonly called "senile" type. Kountz and Alexander found that the lesion in these cases begins in the intervertebral disc. This is manifested as a generalized degeneration with swelling a marked feature. Some of the discs in a given case may be severely involved while others are only moderately so. The earliest change observed is swelling in the region of the nucleus which spreads outward over the annulus lamellosa. This early process may be seen in some of the discs of a given case, while in others the degeneration may be more advanced and the entire disc substance swollen. Upon gross inspection of a disc cut across, large fissures appear between lumps of degenerated tissue. There is usually a ring of fairly healthy annular tissue left around the edge. This corresponds to the epithelial ring. Under the microscope the degenerative process in the cartilage is readily seen. Large fissures are present and also areas of structureless tissue that take an acid stain.

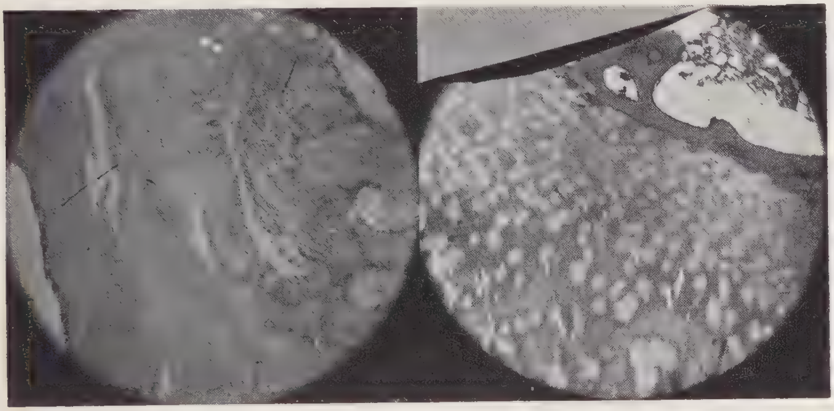


Fig. 10. Sections of Intervertebral Discs

A, cartilage taken from a case of postural emphysema, showing (1) loss of cellular outline, (2) cavities within the substance of the cartilage; *B*, normal cartilage.

In some discs the process may have gone on to the extent that the disc becomes functionless and completely separated from the bone. The cut surface then shows the cartilaginous plate of the vertebral body covered with large areas of degnerated disc remnants. The layers of the annular lamella may be so involved that the still healthy fibers are torn away from the bone at points of degeneration. Calcium deposits are occasionally observed in the degenerated discs and these may be recognized in x-ray films. Hemorrhage into the discs with pigmentation may occur. Eventually, the fibers and disc substance undergo complete dissolution. The recent important work of Beadle and of Schmorl on diseases of the intervertebral discs describes these lesions in great detail.

As the degenerative disease of the disc progresses, the vertebral bodies become more and more invaded by the process, and finally thin out. The lesion may reach the stage where the spine can no longer support its burden, and collapse at some point then occurs. This is found in patients in whom the degenerative process is far advanced. The region most commonly affected is the upper thoracic.

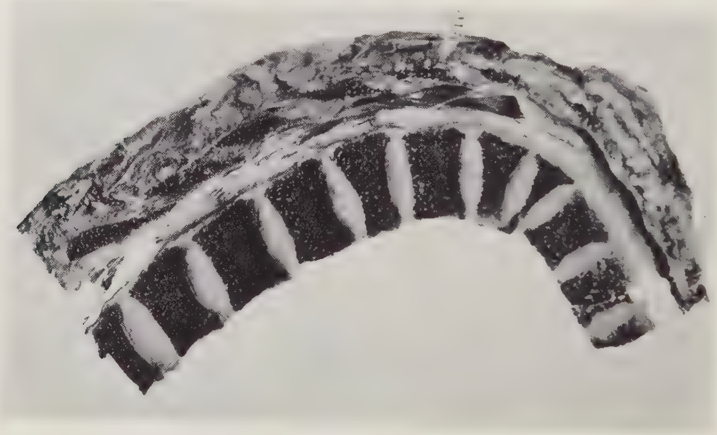


Fig. 11. Section of the Thoracic Spine Showing the Anatomic Lesions of Postural Emphysema, with Collapse of a Vertebra

When the spine collapses, kyphosis results, and the marked hump in these advanced cases of postural emphysema is a familiar clinical picture. When this occurs, the mechanism of the resulting barrel chest is that already described (see fig. 9).

Clinically, postural emphysema may be distinguished from obstructive emphysema. On inspection, in early cases, the straight stiff dorsal spine with obliteration of the posterior thoracic curve is diagnostic of the postural type. This deformity, however, occurs only in comparatively few cases wherein the process of swelling of the discs has just reached the point where the upper vertebral column is straightened, and before collapse has occurred. When a moderate kyphosis is present, it usually is not difficult to distinguish this from the bowing found in obstructive emphysema where all discs and vertebral bodies are usually affected equally, and the spine assumes a continuous curve. In postural emphysema, disease of the discs and vertebral bodies usually is localized to one or more discs and does not involve all. This localization leads to an acute bending where the process is farthest advanced.

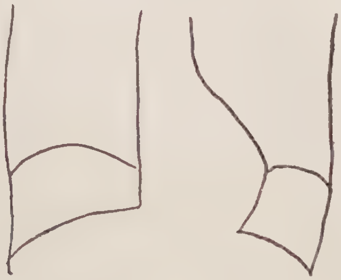


FIG. 12. ORTHODIAGRAM OF MAXIMUM DIAPHRAGMATIC EXCURSION IN A PATIENT WITH POSTURAL EMPHYSEMA

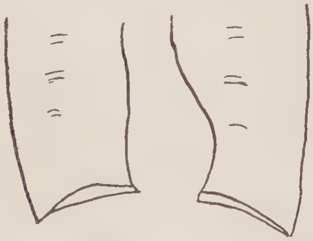


FIG. 13. ORTHODIAGRAM OF MAXIMUM DIAPHRAGMATIC EXCURSION IN A PATIENT WITH ASTHMA AND OBSTRUCTIVE EMPHYSEMA

The patient was of the same general body type as that represented in figure 12

A more reliable differentiation of the two types on inspection is the character of breathing which each presents. In advanced obstructive emphysema, the diaphragm is pushed downward to a flattened position so that it can no longer function, and breathing is entirely costal. In postural emphysema, as the lungs stretch to follow the development of a barrel chest, the horizontal rather than the vertical diameters are increased. The diaphragm therefore remains in its normal position and abdominal breathing is readily apparent. These effects are best elicited with deep respiration. The differentiation between the two types becomes difficult when neither is advanced and both costal and abdominal breathing appear in each.

Fluoroscopic examination of the chest brings out the distinction more clearly, for the diaphragmatic excursion may be measured. It is interesting that whereas in the obstructive type the diaphragm tends to be flat, in postural emphysema the dome may be arched more than normal. This effect is accounted for by distortion of the lungs in that they appear to be stretched horizontally at the expense of their height. As a consequence, diaphragmatic movement is often greater than in normal individuals. The maximum excursion observed was 16 cm.

The activity of the abdominal and chest muscles in the two types of emphysema has been recorded graphically. Rubber tubes of sturdy wall construction were placed around the chest at the level of the junction of the fourth rib with the sternum, and around the abdomen 6 cm. below the xyphoid cartilage. Closed circuits were made by joining the ends of the tubes. They were connected to rubber tambours, whose movements were recorded on a kymograph, and the apparatus was standarized so that there was a constant ratio between the force exerted on the tubes and the excursion on the drum. Simultaneous records were taken of abdominal and chest movements. The patients inhaled through a spirometer, and a measured amount of air was taken into the lungs. Quiet, moderate and deep respirations were recorded.

Tracings of respiratory movements in obstructive emphysema showed a definite preponderance of activity of the chest wall compared to that of the abdomen. Even with attempted deep inspiration, there was little abdominal excursion. Conversely, the tracings of patients with postural emphysema demonstrated increased excursion of the abdominal muscles in contrast to diminished chest movement.

Engelhart, in a study of respiratory motions, has obtained similar rcords in senile emphysema. When the tracings of both types of emphysema were compared to those obtained from the normal individual, one found that in postural emphysema the abdominal activity was greatly increased, whereas in the obstructive type the thoracic excursion was greater than in the normal. As is well known, the movement of the chest compared to that of the abdomen varies in normal individuals, but the ratio is roughly one to one.

Kountz and Alexander compared further functional studies in each type of emphysema. These included observations of the vital capacity, the O_2 and CO_2 content of the arterial blood, the venous and arterial blood pressures, and response to increased CO_2 in the inspired air (tables 5 and 6).

The vital capacity was reduced in each type of advanced emphysema. It was, however, relatively more reduced in the obstructive than in the postural form. In the cases recorded in the tables, the percentage of normal in obstructive emphysema averaged 55, while in the postural type it was 85. The arterial blood pressure is usually low in obstructive emphysema, but apt to be elevated in the postural type, which occurs particularly in older people, and an associated arteriosclerosis is common. The venous pressures were found to be increased in obstructive emphysema, but normal in the postural form. In obstructive emphysema the oxygen content of the arterial blood was low, even with the patient at rest. The CO_2 content was usually increased. In contrast to this, the oxygen saturation of the arterial blood was recorded always as normal in postural emphysema, and likewise the CO_2 concentration was found to be well within normal limits. The tolerance for increased CO_2 in the inspired air in patients with each

TABLE 5

Obstructive emphysema

PATIENT NUMBER	VITAL CAPACITY	PER CENT* OF NORMAL VITAL CAPACITY	ARTERIAL BLOOD PRESSURE	VENOUS PRESSURE	OXYGEN CONTENT ARTERIAL BLOOD	PER CENT OF SATURATION OF ARTERIAL BLOOD WITH O_2
	cc.			*cm. water*	*vols. per cent*	
1	1,300	52	110/80	16	14.2	77
2	1,700	50	105/72	8	16.7	87
3	2,400	60	112/75	12.2	17.7	
4	2,200	58	120/80	8.5	16.8	90
5	2,500	55	107/60	7.2	15.2	
6	2,200	48	130/80	12	15	86.4
7	1,800	48	105/72	9.5	14	82
8	1,700	43	115/85	10.7	16	90
9	2,600	66	160/90	9.5	16.5	85
10	2,700	72	130/85	13.5	16	90

* Based on Dreyer's Tables.

type of emphysema was observed, and it was found that patients with postural emphysema could not stand a high concentration of CO_2 tolerated by those with the obstructive form.

From these studies it is apparent that postural emphysema leads to little impairment of respiratory function. This fact supports the contention that the disease is not essentially a pulmonary disorder excepting in advanced cases where anatomical lesions occur because the lungs have been overstretched. Even in such instances the lesion is frequently localized to the area of the thorax, where widening is greatest and the remainder of the lung compensates for the portion where function is impaired.

TABLE 6

Postural emphysema

PATIENT NUMBER	VITAL CAPACITY	PER CENT* OF NORMAL VITAL CAPACITY	ARTERIAL BLOOD PRESSURE	VENOUS PRESSURE	OXYGEN CONTENT ARTERIAL BLOOD	PER CENT OF SATURATION OF ARTERIAL BLOOD WITH O_2
	cc.			*cm. water*	*vols. per cent*	
1	3,200	89.9	160/90	5	18	96
2	3,400	90.8	140/85	4.5	17.4	
3	3,800	92	168/78	4.5	17.2	95
4	3,600	85	205/98	6	18.2	94
5	3,300	87.4	165/87	8.2	17.6	93
6	2,600	75.2	175/100	5.5	18	94
7	4,000	100	140/87	4		
8	3,200	87	175/105	4		
9	3,700	90	160/90	4.4		
10	3,900	96	127/82	5.4	18	94.2
11	2,700	80	154/98	4.8		
12	2,800	85	120/80	5		
13	3,300	90	132/82	5.5	17.8	95
14	3,500	85	210/120	5		
15	3,600	84	196/105	5.5		
16	3,900	92	187/99	5.8	18	95.2
17	4,000	102	130/87	4.8	18.1	94.3

* Based on Dreyer's Tables.

The question whether senile degenerative changes of pulmonary tissue may play a part in the development of postural emphysema in some instances cannot be answered definitely. At most, the rôle is probably not often an important one, since it does not lead to an extensive loss of elasticity. This is manifested by the prompt collapse of the lungs when the chest is opened, excepting in cases where there is much kyphosis.

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OBSERVATIONS ON THE ETIOLOGY AND TREATMENT OF ANEMIA ASSOCIATED WITH HOOKWORM INFECTION IN PUERTO RICO[1]

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CONTENTS

I. Introduction 318
II. Methods 319
III. Observations Concerning the Etiology of the Anemia 321
  A. Morphological Considerations 321
    1. The Blood Picture 322
    2. The Bone Marrow 325
  B. Physiological Considerations 326
    1. The Relation of the Hookworm to the Anemia 326
      a. The Possible Toxic Effects of the Hookworms 327
        The Effect of the Removal of the Hookworms upon Blood Production 328
        The Effect of Hematopoietic Substances upon Blood Production without Removal of the Hookworms 333
      b. The Effect of Blood Loss 340
    2. The Relation of Factors Other than the Hookworm to the Anemia .. 343
      a. The Relation of Dietary Defects to the Anemia 343
      b. The Relation of Gastrointestinal Pathology to the Anemia ... 349
IV. Observations Concerning the Treatment of the Anemia 352
  A. The Effect of Removing the Hookworms 352
  B. The Effect of Iron Salts 352
  C. The Effect of Various Extracts of Liver 354
  D. The Effect of Improved Diet 360
V. Observations in the Field 360
VI. Discussion 366
VII. Conclusions 370
VIII. Bibliography 372

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[1] These observations were carried out in 1931 by the Commission of the Rockefeller Foundation for the Study of Anemia in Puerto Rico.

I. INTRODUCTION

Since the demonstration of the widespread occurrence of hookworm infection in the Southern United States by Stiles (1), Harris (2), and others (3) (4), the presence of hookworms in the intestinal canal of thousands of anemic children and adults in certain areas of the New World has been established. Although the exact causal relationship of the parasite to the anemia has not been made clear, hookworm disease in regions of severe infection has been regarded in a general way as synonymous with anemia when the latter was not due to other obvious causes. The very name given to the parasite, *Necator americanus* (American murderer), symbolized the general belief that in this widespread anemia a living parasitic agent was primarily the cause of disability and death. Although certain observations have been made which do not conform entirely to the simplicity of this idea, only in the past decade has the relationship of defects of nutrition to certain types of anemia been demonstrated in any quantitative manner. Recently the idea of a relationship between malnutrition and the degree of anemia in hookworm disease has been suggested by Smillie (5), Schapiro (6) and others.

The influence of these two points of view concerning the etiology of the anemia of hookworm disease may be seen to advantage in the history of anemia in Puerto Rico, where it has long been noted to be a common affliction. In 1788 Abbadd (7) stated "The jibaros (peasants) lacked liveliness and had the color and aspect of convalescents." In 1883 Del Valle (7) remarked "It is only the exception to see one of good ruddy color." At this time, according to Ashford (8), the anemia was commonly believed to be due to faulty diet. Therefore, the popular conception, although not technically so expressed, was that of a dietary deficiency anemia. Somewhat later as generous food with relatively small doses of iron and arsenic apparently failed to cure, the condition was attributed to malaria, climate or lack of hygiene. In 1888 Blanchard (8), referring to the finding of hookworms in other West Indian islands and in Brazil, suggested that the parasite was probably present in Puerto Rico as well. In 1899 Ashford (7) discovered the parasite in Puerto Rico. Five years later Ashford, King, and Igaravídez (8) published an extensive report of their observations upon "anemia" in Puerto Rico. In the report they stated, "We have seen that the major portion of this 'anemia' is uncinariasis, and we earnestly recommend to our professional brethren . . . . that no further reliance be placed on iron, quinine, and tonics in general. . . . ." They also reported the failure of improved diets in the relief of anemia. In this way "anemia" in Puerto Rico, as in other regions of hookworm infection, became "uncinariasis." The study of the problem then became for several years the study of appropriate measures against the parasite.

However, in 1929, perhaps influenced by the recent changes in point of view toward the mechanism of anemia elsewhere, Suárez, Otero, and Molina (9) stated in a report of observations upon two cases of anemia that the most important factor in the production of anemias in Puerto Rico was the inadequate diet. It was only then emphasized that Blaud's pills had long been used in Puerto Rico as an adjunct to measures against the hookworm. Moreover, in 1931 Suárez (10) observed a reticulocyte response in a case of hookworm anemia following the administration of an aqueous extract of liver. Although unaware of these particular facts, it seemed worth while to us to attempt to resurvey the problem of the anemia of hookworm disease in Puerto Rico in the light of recent observations upon the etiology and treatment of similar types of hypochromic anemia not associated with the hookworm. To the attack upon the disease by the elimination of the parasites and by the prevention of their spread, it was hoped that a third method might be added—the prevention or treatment of the anemia per se. It was our good fortune to be able to carry out the observations described below, of which a preliminary report has already been published (11), under the auspices of the Rockefeller Foundation in 1931.

II. METHODS

Studies were made of 83 patients with anemia and hookworm infection. The patients, who were mostly from rural districts, were observed either in various hospitals or as regular visitors to a special clinic established at the Presbyterian Hospital in San Juan. The patients were primarily selected because of the severity of their anemia and the absence of complicating infections or sources of blood loss. Children, as well as adults of both sexes, were included in the series. In addition, field observations were carried out by Miss Celia Nuñez at Cidra, a small agricultural community, upon 32 patients, both children and adults, whose anemia was associated with hookworm infestation. The methods and results of this work, by permission of Miss Nuñez, are briefly described below, and are to be published elsewhere in full (12).

Patients under observation in the hospitals, unless otherwise specified, were maintained on a basal diet resembling as closely as possible the usual food of the Puerto Rican peasants. The major articles of this diet were rice, beans, and white bread, such tropical vegetables as yautiá, ñame and plátano, and fruit juices. Meat and eggs were not given. A small amount of milk was allowed in coffee. Salt and sugar were given as desired. Carbohydrate desserts with syrup were freely used. The quantity of the diet was limited only by the desire of the individual for food. In the management of out-patients, suggestions as to diet were never made. In as many instances as possible a general history and diet history were taken, and a physical examination was performed. The blood was examined for malaria in all doubtful cases, and the Kahn test was performed in all instances. The presence of hookworm was assured by direct examination of the stool for ova. In certain instances counts of the ova in the stools were made by the Stoll technique. In others all the worms expelled by efficient anthelmintics were collected. These essential and time-consuming observations were kindly performed by Dr. Florence King Payne. On 54 patients gastric analyses were performed.

The hematological studies, except in the field experiment referred to above, were made from 5 cc. samples of venous blood drawn without stasis and rendered incoagulable by 0.05 cc. of a 20 per cent solution of potassium oxalate. Since the dilution and cell shrinkage factor was small and constant, no correction was made for it. The corpuscular counts were made by the usual technique with certified apparatus. The hemoglobin percentage was determined with a single Sahli instrument with a solid standard. One hundred per cent of hemoglobin was considered equivalent to an oxygen capacity of 21 volumes per cent. The mean corpuscular volume and hemoglobin indices were calculated from hematocrit values determined by the method of Wintrobe (13). The serum color was estimated against bichromate standards by the method of Murphy (14) and the serum bilirubin was determined by the quantitative Van den Bergh method. Blood films were made upon cover glasses and supravitally stained for reticulocytes with brilliant cresyl blue before being counterstained with Wright's stain. When patients were in hospital, a 5 cc. sample of venous blood was usually taken every other day, and reticulocyte counts were made daily from capillary or venous blood as was most convenient. Out-patients usually had venous blood samples examined every one or two weeks. Biopsies of the sternal bone marrow were made upon 15 patients at various stages of their anemia.

Aside from these purely descriptive observations, the general plan of study was to observe under controlled conditions the effect, especially upon blood formation, *of a single procedure*. Thus, individually, the effect was observed of removal of the parasites of the administration of a high-protein diet, of various extracts of liver and of iron salts. The immediate effect of these procedures upon blood formation was determined by daily observations of the reticulocyte percentage, a method which has been applied to the study of both macrocytic and hypochromic anemias (15) (16). In addition, the effect of any given procedure upon the red blood cell and hemoglobin values was determined. Certain of the therapeutic procedures mentioned above were carried out in the field experiment at Cidra, where observations of the red blood cell and hemoglobin values were made upon capillary blood.

III. OBSERVATIONS CONCERNING THE ETIOLOGY OF THE ANEMIA

A. Morphological considerations. At the same time that the study of the anemia of hookworm disease was under way, observations were being made upon sprue as well as upon other types of anemia encountered in the same community. It became apparent that in certain instances patients with hookworm ova in the stools presented the clinical and hematological picture of sprue. Conversely, patients diagnosed clinically as sprue, sometimes presented a blood picture indistinguishable from that associated with severe hookworm infestation. These facts indicated that it was not easy to discriminate between the two conditions in all instances. Furthermore, several patients with no hookworm infection were observed to show blood pictures and hematological responses identical with those of patients with hookworms. The 83 patients forming the basis of this report were thus of necessity selected somewhat arbitrarily because they showed a severe degree of hypochromic anemia and had hookworm ova in the stools.

1. The blood picture. The blood findings in the 83 patients on first coming under observation are summarized in table 1. The outstanding features of the anemia were the reduction of the average cell size and hemoglobin concentration. Thus, the hemoglobin values were reduced in proportion to the number of red blood cells. Twenty-four patients had a color index of less than 0.5; only 9 had a color index of over 0.7. The severity of the anemia of the patients selected may be judged from the fact that only 5 patients had initial hemoglobin values of 50 per cent or over. Of the remaining 78 patients the initial hemoglobin values were less than 10 per cent in 3, between 10 and 19 per cent in 7, between 20 and 29 per cent in 26, between 30 and 39 per cent in 30, and between 40 and 49 per cent in 12 patients. According to Wintrobe, the average normal value for the mean corpuscular erythrocyte volume is 87 cubic micra with limits of 80 and 94 cubic micra. In this series, 7 patients had initial values within normal limits, but the majority showed a distinct reduction. Fifteen patients had mean corpuscular volumes of 60 cubic micra or less when first observed. Under controlled conditions the reticulocytes were usually less than 3 per cent, but in some patients without therapy reticulocyte counts varying from 3 to 6 per cent were found over periods of several days. Rarely an isolated higher value was noted. Nucleated red blood cells of the normoblast type occasionally were seen in the blood films of the patients with the more severe degrees of anemia. The number of leucocytes was usually within normal limits or slightly increased. Occasionally leucopenia with relative lymphocytosis was observed. Eosinophilia was common. Platelet counts were not made, but the impression gained from observation of blood films was that no significant variation from the normal existed. The icteric index was with few exceptions below 6, the normal value for the method. This fact was confirmed by the quantitative Van den Bergh test.

TABLE 1

The blood findings, number of ova or hookworms, results of gastric analyses, and incidence of glossitis and diarrhoea

CASE NUMBER	AGE	SEX	RED BLOOD CORPUSCLES	HEMOGLOBIN	WHITE BLOOD CORPUSCLES	RETICULOCYTES	ICTERIC INDEX	COLOR INDEX	MEAN CORPUSCULAR VOLUME	OVA PER CENTIGRAM	NUMBER OF WORMS	GASTRIC ANALYSES*	GLOSSITIS	DIARRHOEA
1	9	M.	3.91	35	7.8	1.6	2	0.45	55.4	+				
2	10	F.	1.83	21	6.8	4.9	2	0.57	66.6	281		Alc.	0	+
3	35	F.	2.22	37	7.3	5.6	1	0.83	85.1	+				
4	33	F.	3.16	35	3.4	1.0	2	0.55	81.4	+				
5	35	M.	3.63	50	5.5	1.4	2	0.69	87.2	50		Alc.	0	++
6	21	F.	4.58	54	4.2			0.59	65.0	112		Alc.	0	0
7	15	F.	4.14	44	8.0	0.6	3	0.53	60.6	+		0		
8	22	M.	2.53	32	6.9	5.2	2	0.63	73.7	357		Alc.	0	±
9	25	M.	2.36	29	4.6	2.7	10	0.61	69.0	201		Hist.	0	0
10	45	M.	1.93	27	8.3	2.4	4	0.70	84.8	+			0	0
11	18	M.	2.29	37	4.7	1.9	2	0.81	56.0	119		Alc.	0	+
12	12	F.	2.85	56	5.3	0.4	6	0.98	97.3	+	102	Hist.		
13	48	M.	3.53	31	5.4	2.0	4	0.44	55.7	+	1,098	0	0	+
14	25	M.	3.76	46	6.6	0.8	3	0.61	64.8	2		0		
15	32	M.	2.48	28	14.1	2.6	1	0.56	65.3	+				
16	12	F.	2.42	22	11.0	1.8	4	0.45	57.6	44		Alc.		
17	12	M.	0.95	10	11.4	2.4	4	0.53	65.2	+	630	Alc.		
18	10	M.	2.37	22	6.8	2.6	3	0.46	63.2	+	344	Alc.		
19	20	F.	2.67	22	9.6	1.4	1	0.41	52.4	394				
20	6	F.	2.80	23	5.7	2.2	3	0.41	63.2	4		Alc.	0	+
21	22	M.	3.60	28	6.5	1.6	1	0.40	54.1	+				
22	19	F.	2.11	18	7.6	2.4	2	0.43	57.8	+		Alc.	0	0
23	51	M.	2.14	24	3.8	1.2	3	0.56	70.0	48		Alc.	0	0
24	20	F.	3.78	49	6.4		2	0.65	67.5	+				
25	40	F.	3.07	50	8.9	1.6	3	0.81	83.3	+		Hist.	+	++
26	9	M.	1.22	9	6.9	0.6	2	0.37	61.4	494			0	+
27	30	M.	2.09	22	1.9	6.4	4	0.53	70.9	286		Hist.		
28	42	F.	3.98	59	6.1	1.0	3	0.74	76.1	+				
29	55	M.	2.91	25	5.6	2.6	2	0.43	57.0	158		Hist.	0	+
30	10	M.	0.86	9	7.0	2.4	2	0.52	63.7	1,307		Alc.	0	0
31	42	M.	3.12	36	5.9	1.0	2	0.58	65.4	625				
32	13	M.	2.00	19	3.0	1.8	2	0.48	56.5	918		Alc.	0	0
33	60	M.	3.03	35	5.6	1.6	2	0.58	64.3	109			+	0
34	63	M.	3.40	43	11.2	1.8	1	0.63	67.6	2		Alc.	0	+
35	16	M.	3.18	46	5.1	2.8	3	0.72	74.2	+		Alc.	0	0
36	10	M.	1.76	18	7.2	0.8	2	0.51	60.2	130		Hist.		
37	11	M.	0.78	8	7.1	2.2	1	0.51	83.3	498		Hist.	0	+
38	9	M.	3.29	42	12.7	5.4	3	0.64	68.1	+				
39	40	M.	1.91	17	2.9	2.4	2	0.45	62.1	375		0	0	0
40	37	F.	2.71	23	4.3	1.6	2	0.42	54.2	362				
41	63	M.	3.53	35	8.0	2.2	3	0.50	61.5	161		0	+	+
42	42	F.	2.58	25	5.4	0.8	2	0.48	54.8	+				

TABLE 1—*Concluded*

CASE NUMBER	AGE	SEX	RED BLOOD CORPUSCLES	HEMOGLOBIN	WHITE BLOOD CORPUSCLES	RETICULOCYTES	ICTERIC INDEX	COLOR INDEX	MEAN CORPUSCULAR VOLUME	OVA PER CENTIGRAM	NUMBER OF WORMS	GASTRIC ANALYSES*	GLOSSITIS	DIARRHOEA
43	45	M.	3.59	35	5.0	1.2	4	0.49	59.7	132		0	0	++
44	50	F.	2.33	23	5.4	1.0	2	0.49	64.8	484			0	++
45	19	M.	2.90	28	15.8	1.6	3	0.48	63.0	360			0	0
46	6	F.	4.25	41	9.2	0.8	2	0.48	53.8	+				
47	10	M.	1.93	26	12.7	9.0	3	0.67	73.5	307				
48	60	M.	2.49	30	7.2	3.4	2	0.60	74.5	306		Alc.	0	+
49	40	M.	2.64	24	6.9	3.0	2	0.45	64.8	+	313	Alc.	+	++
50	12	M.	3.80	38	11.8	2.8	4	0.50	63.4	182			0	0
51	14	M.	3.07	34	7.9	2.2	3	0.55	61.2	250		Alc.		
52	30	M.	2.70	25	6.8	1.0	3	0.46	63.7	655	76+	0	+	+
53	35	M.	2.03	28	5.5	0.8	3	0.69	76.8	931	2,231	Alc.	0	0
54	35	M.	3.66	38	7.7	0.8	3	0.52	64.2	+			0	0
55	32	M.	2.97	31	7.7	2.0	4	0.52	64.2	402				
56	17	F.	3.53	36	7.3	1.4	3	0.51	63.7	+		0	0	0
57	20	M.	3.48	43	9.6	1.2	1	0.62	66.5	+	562	Alc.		
58	38	M.	2.51	25	6.3	4.6	2	0.50	55.6	113	155	Alc.	0	0
59	35	M.	2.43	25	6.4	2.4	4	0.51	71.1	+	1,066	Alc.		
60	12	M.	1.82	20	9.2	3.8	2	0.55	66.9	+	718	Alc.		
61	14	M.	2.02	21	2.8	2.4	4	0.52	61.8	101		0		
62	52	M.	2.14	32	4.4	3.0	3	0.77	68.4	607	1,424	Alc.	0	0
63	19	M.	4.38	38	8.9	1.8	2	0.43	58.2	112	103	Hist.		
64	68	M.	3.57	44	7.4	5.2	2	0.61	71.5	118			0	0
65	18	M.	1.57	22	6.8	1.4	2	0.69	78.4	601		0	+	0
66	40	M.	2.55	35	5.3	0.7	2	0.69	75.6	650	723	Hist.	0	+
67	35	M.	2.60	36	3.1	1.0	2	0.69	76.2	+	724	0		
68	18	M.	1.98	27	10.0	2.8	4	0.68	70.2	+	1,234	Alc.	0	+
69	17	M.	2.88	33	3.8	1.0	3	0.67	67.9	30		Hist.	0	0
70	20	F.	2.77	39	7.1	3.6	2	0.72	73.2	+	1,106	Alc.		
71	19	F.	2.07	37	6.4	1.1	1	0.89	91.8	+		Alc.	+	+
72	18	M.	2.62	19	3.0	1.8	4	0.59	72.7	179		0	0	0
73	28	M.	3.00	36	6.6	0.8	5	0.60	65.6	+				
74	35	M.	3.54	34	4.8	1.6	3	0.48	59.0	+		Alc.	0	0
75	34	M.	2.80	39	4.3	2.2	3	0.70	77.1	+		Alc.	+	+
76	25	M.	4.12	45	6.5	1.0	3	0.55	65.0	+				
77	6	F.	1.77	17	12.1	0.8	3	0.48	59.8	302			0	+
78	15	M.	3.63	34	9.2	1.4	2	0.48	62.7	49				
79	35	F.	3.49	36	4.7	2.0	8	0.52	64.2	+			0	0
80	18	F.	3.68	41	9.2	0.4	3	0.56	62.0	152				
81	24	F.	3.53	38	6.8	3.2	2	0.54	51.4	+	40	0		
82	30	F.	2.88	37	4.5	3.6	4	0.64	66.1	124		Hist.	0	+
83	18	F.	3.71	46	5.7	1.8	4	0.62	63.5	+		Alc.	0	0

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* *Alc.* indicates secretion of free HCl after ingestion of 50 cc. of 7 per cent alcohol; *hist.* after injection of 0.5 mgm. of histamine phosphate; 0 indicates no secretion of free HCl after histamine injection.

With the exception of the mean corpuscular volume determinations in these patients, nothing new is added to the description of the blood picture of the hypochromic anemia of hookworm disease. The blood picture in this series of patients was that of a hypochromic anemia, usually microcytic, without evidence of active blood regeneration. It thus differs in no obvious way from the blood picture of types of hypochromic anemia not associated with hookworm infestation encountered in Puerto Rico and elsewhere.

2. *The bone marrow.* The bone marrow findings are also in agreement with those found in other types of hypochromic anemia. Piney (17) has shown that only the ribs, flat bones, and vertebral column normally contain active cellular marrow in adult life. Peabody (18) has emphasized the fact that the pathological process present in the normally cellular areas does not necessarily extend to the long bones. For these reasons biopsies for histological examination of the bone marrow were performed on the sternum. A specimen of marrow was removed from this site at biopsy at various stages of the anemia in 15 patients, and the tissue subjected to histological study.

The sections were moderately but quite definitely more cellular than the normal marrow. The degree of increased cellularity varied in each section and varied from patient to patient. The number of fat cells was decreased and vascularity was marked. Islands of young cells were present with pale, clear cytoplasm and round to oval nuclei containing a few small, discrete masses of chromatin. These cells were considered to be at a stage not far removed from hematopoietic vascular endothelium. A second cell type was seen, although not in large numbers. This was a small round cell with a narrow rim of cytoplasm and a nucleus containing large masses of heavily stained chromatin. These cells were considered to belong to a primitive pluripotential cell type. The predominant cell type, however, was the normoblast. Large numbers of these cells were present, both in islands and diffusely scattered throughout the tissue. There was a sharp disproportion between erythroblastic cells and those of the granulocyte series. Whereas this ratio is about equal in normal marrow, here the former outnumbered the latter from three to five times. The small number of granulocytes present showed no disturbance of maturation.

The histological picture described differs from the normal and from that of the marrow in cases of severe anemia due to toxic or infectious conditions such as nephritis of severe grade. It resembles to a certain extent the findings of other investigators of hookworm disease. Ashford and Igaravídez (7) reported the finding of many normoblasts and eosinophils. Day and Ferguson (19) occasionally found in addition a gelatinous appearance in the femoral marrow of autopsied patients. Dameshek (20) and others have reported characteristic pathological changes in the bone marrow removed at biopsy from the sternum of cases of idiopathic hypochromic anemia. The marrows are stated to be more cellular than normal, with less fat, and to show an increase in the ratio of erythroblastic to leucocytic cells. Since the histological picture in the bone marrow of the hypochromic anemia of hookworm infestation thus apparently closely resembles the bone marrow picture in hypochromic anemia not associated with hookworm infestation, the common genesis of both types of anemia is suggested. These observations will be discussed in detail in a later communication.

B. Physiological considerations. The belief that the hookworm is the dominant factor in producing the anemia associated with it deserves careful study because, although it has been so often expressed and generally accepted, no entirely satisfactory explanation of its method of action has yet been given. For this reason consideration was given to the ways in which the parasite, alone or as one of several factors, could conceivably have a relation to the anemia.

1. The relation of the hookworm to the anemia. Although in general there is stated to be a relationship between the degree of anemia and the number of worms harbored, attempts at correlation of the number of parasites with the degree of anemia in individual cases have met with no particular success in the hands of others (5) (6) (21) (22) (23). This feature of the analysis was accordingly not exhaustively pursued. However, in 17 patients all the stools were saved and worm counts were made during elimination of the parasites with hexylresorcinol by the technique of Lamson (24). In figure 1 is shown the relationship for 17 individuals between the number of parasites discharged and the initial hemoglobin level. It is clear that there is no obvious correlation. On the same chart are also plotted for 55 individuals the average number of ova in a centigram of feces, corrected for the consistency of the material, against the initial hemoglobin value. Again there is apparently no significant correlation to be observed. In this respect, then, these observations are in agreement with other reports and clearly suggest the influence of factors other than the hookworm. The possibility of a contributing influence nevertheless remains.

a. The possible toxic effects of the hookworms. Although Whipple (25) has demonstrated weak hemolytic factors in the hookworms of men and dogs, convincing evidence for the rôle of hemolysis in the production of this anemia is lacking. The morphological characteristics of the anemia are not those of a hemolytic process, such as is illustrated by hemolytic jaundice, in which increased numbers of reticulocytes and increased serum color are constantly present. It is, of course, possible that such a condition, if originally present, might later be hidden beneath the impress upon the blood picture given by the failure of the bone marrow. However, with the exception of

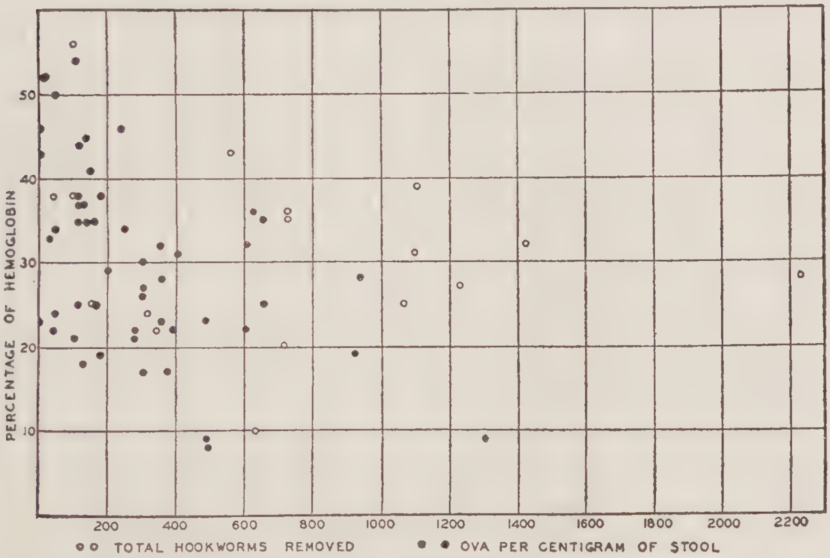

FIG. 1. RELATIONSHIP BETWEEN THE ORIGINAL PERCENTAGE OF HEMOGLOBIN AND THE NUMBER OF WORMS SUBSEQUENTLY REMOVED FROM 17 PATIENTS, AND BETWEEN THE ORIGINAL PERCENTAGE OF HEMOGLOBIN AND THE AVERAGE NUMBER OF OVA PER CENTIGRAM OF STOOL OF 55 PATIENTS

malaria or of a septicemia, particularly that due to the streptococcus, the action of infection is not that of a hemolytic process but of an inhibition of bone marrow function. De Langen (26) champions this theory; and Fülleborn (27) states that the hookworm secretes a substance which has a toxic effect on the bone marrow. If, then, the presence of hookworms has a dominant effect in depressing the bone marrow activity, two phenomena should be demonstrable. First, the removal of the parasites should produce increased blood regeneration, and secondly, the presence of the parasites should inhibit, to a certain extent at least, the influence of agents capable of promoting blood formation. Observations designed to test the validity of these points were accordingly made.

The effect of the removal of the hookworms upon blood production. Twelve patients were kept in hospital upon the basal diet referred to above, and no form of therapy for anemia was given. After a control period of a few days the parasites were removed by means of hexylresorcinol (24) and the effect upon reticulocyte production and red blood cell and hemoglobin values observed. In table 2 are shown the effects upon the hemoglobin and red blood cell values and the number of hookworms removed in each instance. It is evident that during periods of time as long as twenty-eight days after the first treatment for hookworms, very little effect upon red blood cell or hemoglobin values was produced. The average gain in red blood cells in 9 patients (calculated by extrapolation in cases 59, 67, and 81) was only 0.48 million per cubic millimeter in twenty-eight days. In two instances, cases 52 and 63, the red blood cells actually declined slightly in number. The effect upon hemoglobin production was even less striking the average increase in 9 patients being only 4 per cent in twenty-eight days. In two instances, cases 59 and 61, the hemoglobin percentage actually became less. During six weeks the average gain in red blood cells of 7 patients was 0.49 million per cubic millimeter and in hemoglobin was 3 per cent. It is thus clear that the removal of the parasites did not bring about any striking increases in red blood cells and hemoglobin, and thus affords no evidence for the dominant inhibitory effect of the hookworms on bone marrow function. It is important to realize, moreover, that the fact that slight increases in hemoglobin and red blood cell values occurred is not necessarily evidence of increased activity on the part of the bone marrow. Precisely such a result would occur if the amount of blood loss were diminished.

In the light of the observations of Minot and his collaborators (15) (16), as well as from the work of others, the value of the study of reticulocyte production as a measure of bone marrow activity has been clearly established for both macrocytic and hypochromic anemias. This phenomenon should, theoretically at least, be applicable to the problem of detecting whether the removal of possible "toxic inhibition" of the bone marrow caused by the presence of the hookworms would result in increased hematopoietic activity. The principle is best illustrated by the effect of removing the parasites in another tropical disease associated with anemia. The anemia of many cases of malaria is clearly due, at least in part, to the rapid blood destruction dependent upon the activities of the plasmodia. The blood picture is the reverse of that of hookworm disease, since it is characterized by signs of rapid destruction and production of red blood cells, best shown by increases of the serum bilirubin and by persistently elevated reticulocyte counts during the active stages of an early infection. However, it is possible to demonstrate that in addition to the hemolytic process there is, as with other types of infection associated with fever, an actual inhibition of bone marrow function. This is shown by the fact that shortly after the abolition of the fever with quinine an increase of the reticulocytes in the peripheral circulation takes place. This temporary increase can easily be demonstrated in suitable cases of malaria, and is best explained on the basis of the removal of an inhibitory effect.

In figure 2 is shown the contrast between the effect of removing the parasites in a patient with malaria and in a patient with hookworm infestation, case 52. Both patients were severely anemic. In the former, the distinct temporary increase of reticulocytes during ten days following treatment with quinine is in sharp contrast to the absence of any such effect resulting from the elimination of the hookworms in the latter patient. If the hemolytic function alone had been abolished by the removal of the malaria parasite, the reticulocytes would have declined steadily without the temporary increase. In table 2 are shown the results of hookworm removal upon reticulocyte production in 12 patients, including case 52. It is clear that in no instance was there a significant increase of reticulocytes. In con-

TABLE 2

Comparison of effects upon hematopoiesis of removal of hookworms and of administration of ferric ammonium citrate to patients upon basal diet

First periods. Hookworms removed with hexylresorcinol*

	CASE 13			CASE 18			CASE 49			CASE 52			CASE 57			CASE 59			CASE 60			CASE 63			CASE 67			CASE 68			CASE 69			CASE 81		
	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes	Red blood cells	Hemo-globin	Reticulo-cytes
days	*mil-lions*	*per cent*	*per cent*	*mil lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*	*mil-lions*	*per cent*	*per cent*
0	3 39	33	1.6	2 37	20	4.0	2.70	26	1.6	2.40	27	0.6	3.17	42	1.0	2 20	33	3.8	2.03	22	2.4	4.31	38	1.2	3.00	37	1.2	2.32	23	3.2	2.48	32	5.2	3.36	40	1.4
2	3 26	34	2.6	2 05	21	4.8	2.81	26	1.4	3.01	30	1.0	3.35	44	1.2	2.77	29	3.8	1.98	22	5.6	4.54	39	1.0	3.02	38	1.8	2.34	25	3.0	2.53	32	3.4	3.62	44	1.8
4	3 21	32	1.8	1.74	20	6.8	3.10	28	2.8	2.68	25	1.0	3.28	44	1.8	3.02	30	3.0	2.20	25	4.0	4.32	35	1.6	2.56	34	1.8	2.28	25	2 4	2.76	37	5.0	3.15	35	0.6
6	3 20	35	1.0	2.37	20	5.7	3.30	30	3.8	2.98	30	0.8	3.41	45	0.4	2.75	29	2.6	2.19	22	3.8	3.65	32	1.8	3.08	36	0.8	2.21	22	0.8	2.70	34	2.6	3.33	37	1.2
8	3 31	43	2.8	1.96	21	4.8	3.20	32	2.8	3.00	26	1 4	3.59	45	2.4	2.78	34	2.2	2.21	22	2.2	3.81	37	1.4	3.26	35	1.0	2.47	25	1.0	2.57	28	3.2	3.40	42	2.2
10	3.46	36	2.0	2 03	20	2.4	3.39	29	1.4	2.67	55	1.0	3.74	48	1.0	3.06	32	1.8	2.42	22	4.6	4.35	33	2.2	3.08	37	0.8	2.39	25	1.6	2.65	31	3.6	3.40	41	2.4
12	3 41	34	2 2	2.27	22	3.2	2.62	31	2.8	2.97	25	0.4	4.05	46	0.8	3.76	31	1.6	2.20	26	3.8	4.06	38	1.6	2.31	31	1.2	2.40	25	3.4	2.60	34	3.6	3.29	39	1.6
14	3 80	35	2.4	1 96	18	2.0	3 57	30	4 2	2.62	27	0.8	3.52	50	1.0	3.83	33	2.4	2.42	28	3.2	4.06	39	3.2	3.38	36	1.2	2.38	21	2.8	2.44	32	4.2	3.60	40	2.0
16	2 69	37	3 4	2 36	23	1.6	3 78	32	1.8	2.65	24	1.6	3.60	50	1.2	3.01	28	3.0	2.74	25	2.0	3.88	37	1.8	3.09	34	0.8	2.35	23	2.4	2.42	31	3.2	3 60	37	2.0
18	3.59	39	0.8	2 35	19	2.4	3.50	29	2 6	2.71	26	1.2	3.76	53	2.6				2.71	25	3.0	4.00	39	1.6	2.96	37	1.8	2.38	25	2.8	2.64	35	2.8			
20				2.43	23	3.0	3 46	31	3.2	2.81	23	1.2	3.66	49	1.0				2.50	26	3.8	4.00	35	1.2	3.55	41	2.8	2.28	26	2.8	2.73	32	4.2			
22				1.41	21	3.8				2.88	28	0.8	3.84	55	1.0				2.25	21	5.8	3.80	38	1.6												
24	3.52	39	1.0	1.90	21	3.0				2.77	26	0.6	3.61	57	2.6				2 39	22	3.4	3.86	39	2.8				2.00	25	3.8	2.53	30	4.8			
26				1.94	22	2.0				2.46	21	1.0																								
28	3.28	37	1.2	2.38	22	2.4				2.33	25	2.0	3.61	47	2.0													2.94	31	2.4	3.09	35	2.8			
weeks																																				
6	3.62	36								2 53	27	1.8	4.12	50	0.6										3.22	38		3.10	30	1.6	3.25	36	3.0			
8	3.95	41					3.03	27	2.4																											
10	3.89	39					3.09	27	2.0																											
12																																				
14																																				
16													4.31	60																						
18																																				
20	4.23	40	1.6																																	

Second periods. Daily administration 6 grams of ferric ammonium citrate

days																																				
0	4.23	40	1.6	2.38	22	2.4				2.53	27	1.8				3.01	28	3.0	2.39	22	3.4	3.86	39	2.8	3.22	38										
2				2.10	22	6.4				2.98	32	6.4				3.39	34	7.2	2.52	31	9.8	3.97	34	3.2												
4				2.40	28	*10.8*				2.98	29	8.0				3.60	36	6.8	2.74	30	*13.4*	4.44	45	5.4												
6				2.70	31	7.0				3.32	35	*11.8*				3.78	44	*10.8*	3.14	36	6.4	4.51	45	6.8												
8				2.46	25	3.2				3.49	39	7.0				4.10	46	9.0	2.87	32	2.8	4.84	42	5.4												
10				2.74	30	2.6				3.59	38	4.2				4.47	51	8.6	2.99	38	2.2	4.86	45	*7.0*	3.11	36										
12										3.68	43	3.2										4.61	51	4.0												
14	4.81	48	2.8							3.96	44	5.0				4.68	55	5.0							3.73	39	2.0									
16																																				
18										3.87	47	3.8																								
20																4.37	60								3.65	54	2.4									
22	5.12	55	1.2																																	
24																																				
26																																				
28	5.02	61	1.6																						4.08	59	1.0									
weeks																																				
6	5.30	68	0.8																						4.39	65										
8	5.31	80																							4.67	72										
Number of worms removed . . .	1,098			344			313			764			562			1,066			718			103			724			1,234			1,106			40		

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* Number of hookworms removed from each patient is given at bottom of table.

trast to this was the positive effect of the subsequent daily administration of 6 grams of ferric ammonium citrate to 5 of these patients. Characteristic reticulocyte responses attended by rapid increases of red blood cell and hemoglobin values appeared in each instance.

It is thus clear that the bone marrow in at least 5 of these patients was in a condition to produce reticulocytes in response to the administration of hematopoietic substances, and therefore could potentially have so responded as a result of the removal of hypothetical inhibitory substances abolished with the hookworm elimination. The negative effect of the removal of the hookworms upon hemoglobin production in cases 13 and 67, in contrast to the striking effect of iron therapy, is graphically portrayed in figure 3. Over periods of 118 and forty-six days, respectively, after the beginning of the hookworm elimination, no

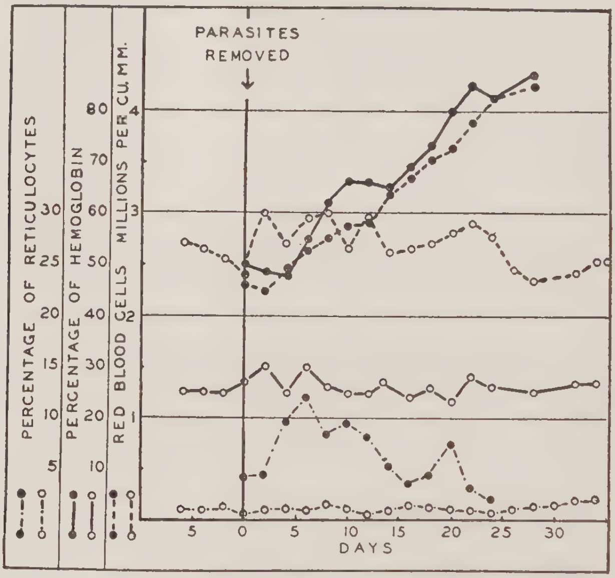

Fig. 2. Comparison of the Effects upon Hematopoiesis of the Removal of the Parasites in a Patient with Anemia Associated with Malarial Infection (Solid Dots), and in a Patient with Anemia Associated with Hookworm Infection, Case 52 (Open Dots)

After abolition of the parasites, note the increase of reticulocytes in malaria, and the absence of such effect in hookworm disease. Note also the rapid improvement of the blood values in the anemia of malaria, and the absence of improvement in the anemia of hookworm disease.

significant increase of hemoglobin was noted. These results were further confirmed by the field observations at Cidra.

The effect of hematopoietic substances upon blood production without removal of the hookworms. The second of the two criteria of the possible influence of the parasite in inhibiting bone marrow function was stated to be a determination of the effect of the presence of the parasite upon the production of blood in response to the administration of hematopoietic substances. If the presence of the parasites produced in some way an inhibition of the bone marrow similar to that of other infectious agents, the depressing effect upon the reticulocyte production and increases of red blood cells and hemoglobin should theoretically be detectable. For this reason the response of the hypochromic anemias associated with bacterial infection to the administration of hematopoietic substances is disappointing in contrast to the effect of such substances in similar types of anemia not associated with infection. The inhibitory effect of infection is also occasionally demon-

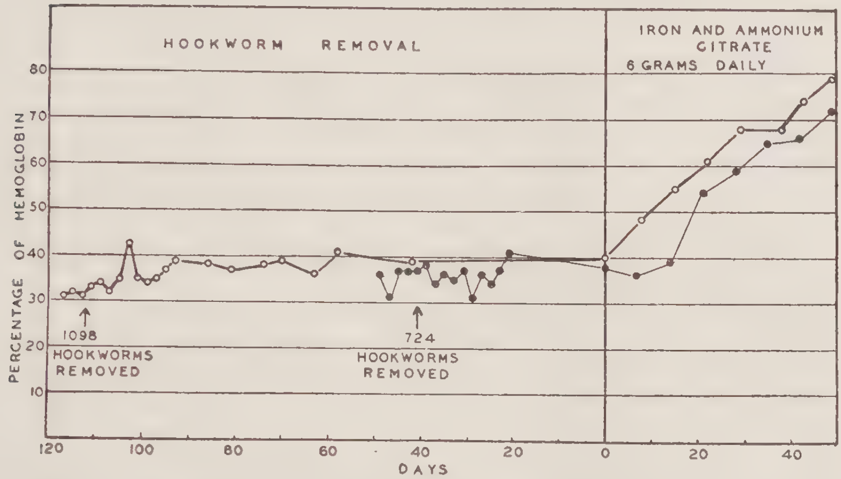

Fig. 3. Comparison of the Effects upon Hemoglobin Production of the Removal of the Hookworms and of Therapy with Iron

Note the negative effect upon hemoglobin production during a period of one hundred and ten days in case 13 (open dots), and during a period of forty days in case 67 (solid dots), following the removal of the hookworms. Compare the striking subsequent effect on hemoglobin production of the administration of 6 grams of ferric ammonium citrate daily.

strable in the treatment of macrocytic anemias. For example, in certain instances in which a reticulocyte response and increase of blood values are progressing in response to the administration of potent extracts of liver, an intercurrent infection has been observed to diminish at once the reticulocyte production and to bring to a halt the increase of red blood cells. Moreover, immediately after the cessation of the fever caused by the infection, the reticulocytes have been observed to rise to their former values and the remission has proceeded in the usual manner.

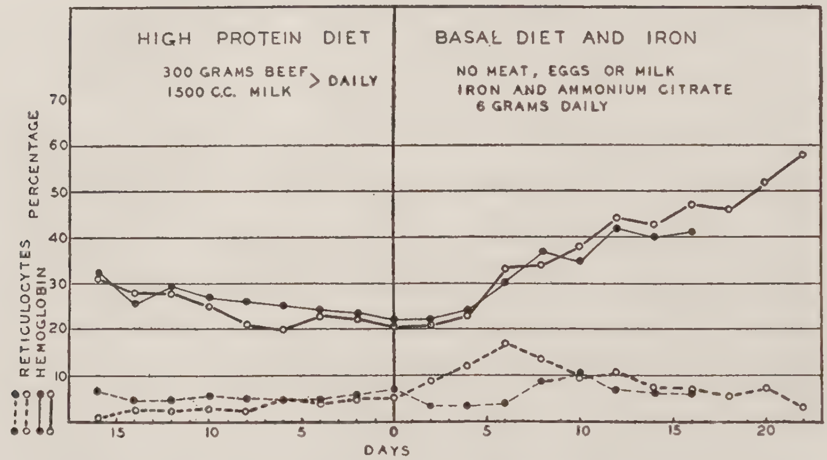

Fig. 4. Negative Effect upon Hemoglobin and Reticulocyte Production of the Daily Addition of 300 Grams of Meat and of 1500 cc. of Milk to the Basal Diet, Contrasted with the Positive Effect of the Daily Addition of 6 Grams of Ferric Ammonium Citrate to the Basal Diet

The results are shown in 2 patients, case 53 (open dots) and case 62 (solid dots). No therapy for hookworms was given.

Accordingly, in order to determine the inhibitory influence of the parasites, the effect of iron administered without removing the hookworms was observed as well as the effect of iron administered after the removal of the hookworms. If a dominant effect of the presence of the parasites was an inhibition of blood production, it should be apparent through a diminution of the reticulocyte responses and increases of hemoglobin and red blood cells resulting from the administration of hematopoietic substances. In table 3 are shown in detail the hematological data for 10 patients maintained in the hospital upon basal diets. No treatment was given for the elimination of the hookworms. During the first periods of cases 5, 9, 11, 16, 47, 69 and 74, either no therapy or ineffective therapy with Liver Extract No. 343, N. N. R., was given. In cases 23, 56, and 66 slight reticulocyte responses occurred as a result of the daily intramuscular injection of 100 mgm. of ferric ammonium citrate. During the second periods of observation 6 grams of ferric ammonium citrate were given daily by mouth to each patient. As a result, prompt reticulocyte responses occurred in 7 of the 10 patients and in all, the hemoglobin and red blood cell values progressively increased. The data in table 4 summarize the effect of iron upon hemoglobin regeneration in 27 patients including the 10 patients referred to in table 3, with initial hemoglobin values of less than 50 per cent. The 17 additional patients were not maintained under constant observation in the hospital, but were given daily 6 grams of ferric ammonium citrate and observed at intervals of one or two weeks in the out-patient clinic. No treatment for the removal of the parasites was given, and no changes of diet were suggested. For convenience the data are presented in a simplified form by plotting upon coördinate paper against days the actual determinations of the hemoglobin percentages made in each case. From the curves so constructed the values for each patient at ten-day intervals were read off and, together with the average hemoglobin values for the group, are shown in table 4. The average increase of hemoglobin during the first thirty days in these 27 patients was 25 per cent.

The influence of identical amounts of iron on hemoglobin production was also observed in 6 patients who had previously had their hookworm burden eliminated. The hemoglobin values were determined at ten-day intervals as for the group of 27 patients just described. The individual and average hemoglobin values are given in table 4 for comparison with those of the patients whose hookworms had not been eliminated. While the average increase of hemoglobin during the first thirty days of the administration of iron, 31 per cent, is apparently greater in this group of patients whose hookworm burden had been removed, it is impossible to be certain that a real difference is involved, because of the small number of patients in this group. The results of the observations in the field experiment at Cidra upon the effect of the daily administration of 6 grams of ferric ammonium

TABLE 3

Effect upon hematopoiesis of daily addition of 6 grams of ferric ammonium citrate (second periods) to basal diet without removal of hookworms

	LIVER EXTRACT NO. 343 N.N.R. DERIVED FROM 10 GRAMS OF LIVER INTRAMUSCULARLY									NO THERAPY			FERRIC AMMONIUM CITRATE, 100 MGM. INTRAMUSCULARLY			NO THERAPY			FERRIC AMMONIUM CITRATE, 100 MGM. INTRAMUSCULARLY						NO THERAPY					
	Case 5			Case 9			Case 11			Case 16			Case 23			Case 47			Case 56			Case 66			Case 69			Case 74		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
First periods. Either no therapy or daily administration of various substances as indicated																														
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	3.63	50	1.4	2.36	29	2.7	2.29	37	1.9				2.76	28	2.6				3.53	36	1.4	3.15	34	1.6	2.88	33	1.0			
2	3.79	47	1.4	2.89	33	2.6	2.29	22	1.1				2.46	26	2.8				3.50	38	2.4	2.65	33	1.6	3.02	32	1.4			
4	3.64	45	1.6	2.89	33	0.4	2.42	23	2.4				2.39	24	*6.0*				3.55	38	2.0	2.73	35	2.1	3.12	35	1.0			
6	3.45	45	3.0	2.74	34	1.8	2.53	21	3.8				2.37	25	4.2				3.39	41	*3.4*	3.11	36	*6.6*	—	—	—			
8	3.85	46	2.0	2.44	30	1.2	1.96	20	3.4				2.53	25	4.8				3.34	40	3.2	3.14	38	4.6						
10	3.29	46	1.8	3.17	34	4.0	1.91	21	1.4				—	—	—				—	—	—	—	—	—						
12	—	—	—	2.98	32	2.4	—	—	—																					
Second periods. Daily oral administration of 6 grams of ferric ammonium citrate																														
0	3.29	46	1.8	2.98	32	2.4	1.91	21	1.4	2.42	22	1.8	2.53	25	4.8	1.93	26	9.0	3.34	40	3.2	3.14	38	4.6	3.12	35	1.0	3.54	34	1.6
2	3.38	44	3.1	3.40	37	2.6	2.16	25	5.8				2.76	27	5.6				3.73	41	5.2	3.53	43	3.6	3.00	37	1.2	3.48	35	1.8
4	3.51	44	4.6	3.03	38	8.0	2.47	25	*13.8*				2.59	30	3.6	2.18	34	13.6	3.81	41	4.2	3.52	44	5.4				3.20	37	2.0
6	3.42	48	6.0	3.00	39	*9.0*	2.58	30	10.8	2.96	34	11.0	2.93	35	4.6				3.86	45	6.6	3.84	46	*6.2*	2.94	41	8.0	3.36	38	2.2
8	3.46	42	4.6	3.24	41	3.6	2.95	33	7.8	3.47	41	*16.4*	2.84	35	4.2	3.11	47	*23.0*	3.76	49	2.9	3.69	44	4.6	3.13	42	*7.2*	3.68	40	2.4
10	3.36	48	4.2	3.14	47	4.6	3.50	40	2.0	3.20	44	6.4	2.94	37	2.4				3.97	50	3.2	3.63	51	1.4	3.80	45	5.6	3.65	41	2.2
12	3.43	54	7.4	3.32	51	2.4	3.65	44		3.65	50	11.8	3.18	37	2.0	3.14	52	5.8	3.87	54	2.0	3.87	50	3.2				3.33	43	1.2
14	3.47	53	7.2	3.93	53	2.0							3.02	38	0.1				4.47	59	*4.0*	3.48	50	1.8	3.56	44	4.4	3.73	44	1.2
16	3.60	58	*9.8*	3.96	56	2.2	3.87	47	3.0	3.56	52	5.4	3.26	41	1.8	3.09	59	3.2	4.17	61	1.6	3.71	53	3.4	3.76	47	3.6	3.83	50	0.4

18	3.76	58	5.6	4.12	58	2.0	3.63	48	2.2				3.15	41	2.8										4.09	48	2.2	3.71	50	0.8
20	3.84	58	3.0	4.11	60	1.8	3.92	53	2.4				3.39	46	1.8				4.20	62	0.8							3.73	52	1.4
22	3.99	62	5.2				3.79	50	4.8				3.28	44	2.4	3.23	53	1.8										3.68	54	1.0
24	4.04	64	1.8				3.90	55	3.2				3.39	48	2.4	—	—	—	4.19	65		3.68	58	2.6				3.47	50	1.4
26	4.15	63	4.6				4.14	62	2.0				3.46	45	2.2										3.77	56		4.02	59	0.8
28	4.30	66	2.2	4.29	64	0.1	3.98	57	2.4	3.37	66	3.6	3.37	53	3.6				4.61	68	0.8	4.00	64	3.0				3.40	56	0.8
weeks													—	—	—													—	—	—
6	4.30	71	4.4	4.39	70	1.4	4.24	64	2.0	3.27	65	2.4							4.83	72	1.0	4.15	76	3.2	4.11	67	1.2			
8	—	—	—	4.73	83		4.44	70	2.2	4.07	71	1.0							—	—	—	4.41	80	0.8	4.73	68	0.4			
10				—	—	—	4.11	68		3.79	73											—	—	—	4.45	73				
12							4.08	57		3.43	74														4.21	70	1.0			
14							—	—	—	3.55	69														4.61	74	0.6			
Ova per cgm. stool	88			201			119			44			48			307			Positive			723 worms*			30			Positive		

---

* Number of hookworms removed after termination of the observation.

TABLE 4

Effect upon hemoglobin production of daily administration of 6-grams of ferric ammonium citrate

CASE NUMBER	HEMOGLOBIN PERCENTAGES AT 10-DAY INTERVALS						
	0 days	10 days	20 days	30 days	40 days	50 days	60 days
	Hookworms not removed						
4	35	38	48	55	63	68	
5	44	48	58	68	71		
7	44	55	64	70	76	83	85
8	34	40	57	63	72	75	83
11	21	40	52	57	61	66	70
16	22	44	54	64	68	70	72
23	25	37	46	53			
24	44	57	66	76			
34	43	53					
37	18	34	41	50	49	52	
38	42	49	57	64	72	77	
39	17	20	26	32	35	41	44
41	36	42	52	56			
42	25	33	43	55	61	67	69
43	35	40	42				
46	41	50	59	65	69	71	73
47	26	47	56				
48	30	40					
56	40	50	62	68	72		
66	40	51	58	62	70	78	80
69	35	45	52	59	64	68	70
71	41	50	55				
72	22	30	41	50			
73	36	50					
74	34	41	51	56			
76	45	58	63				
80	41	52	66	62	70		
Averages....	34	44	53	59	65	68	72
	Hookworms previously removed						
13	40	50	59	68	70	80	
52	27	38	47				
59	33	47	59				
60	22	37					
63	36	48					
67	38	37	52	60	66	71	
Averages....	33	43	53	64	68	76	

citrate fortunately bring additional evidence to bear on the question. In 5 patients whose hookworm burden was removed, during the first thirty days the average gain in hemoglobin (35 per cent) was slightly less than the average gain in hemoglobin (38 per cent) of 7 patients who had received no treatment for the parasites. Each group gained 44 per cent of hemoglobin during the first sixty days of therapy with iron. The hemoglobin percentages for each patient were calculated for ten-day intervals as described above, and, together with the averages, are given in table 9. The average hemoglobin values for each group are also plotted at ten-day intervals in figure 5. It is reasonable to conclude, therefore, that at least no evidence for a significant inhib-

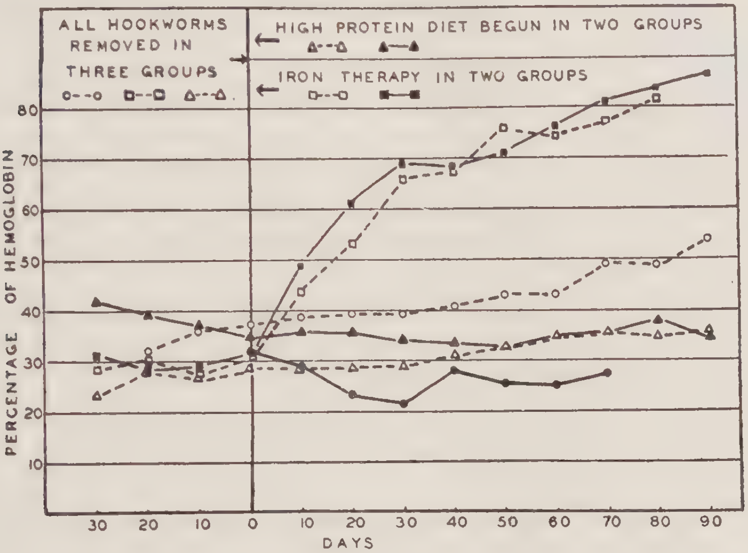

Fig. 5. Comparison of the Effects of Removal of the Parasites, of Improved Diets, and of Therapy with Iron. Results of Observations in the Field at Cidra on the Average Hemoglobin Production in 6 Groups of Patients

The 32 patients were divided into two main groups, A and B, with three subgroups each, I, II and III respectively. The 16 patients of group A (open symbols) had the hookworms removed before the beginning of the experimental period (vertical line in figure). The 16 patients of group B (solid symbols) received no treatment for hookworms. The patients of groups A-I and B-I (circles) had no change in diet. The patients of groups A-II and B-II (triangles) were given as an addition to their usual diet 300 grams of meat and 1500 cc. of milk daily. The patients of groups A-III and B-III (squares) had no change in diet, but received daily 6 grams of ferric ammonium citrate. Note the positive effects of iron in groups A-III and B-III in contrast to the negative or slight effects of removal of the hookworms or improvement of the diet.

itory effect of the parasites upon the bone marrow was demonstrable by our observations.

b. The effect of blood loss. It is clear from the experiments of Wells (28) that at least in the dog considerable amounts of blood are removed by the hookworm. He estimated that in twenty-four hours 100 hookworms removed 84 cc. of blood. Although Smillie (29) states that a large amount of blood is never seen in the intestinal tract of *Necator*, it is reasonable to suppose that some blood loss does occur as the result of infection with this as with other species. According to Bass (30) tests of the feces for occult blood are nearly always positive, even in mild or moderate infections. In our experience hemorrhagic spots were observed at the point of attachment of the worms in the intestinal tract of autopsied patients, and the worms would ingest red blood cells after being placed in saline solution.

The anemia of acute blood loss is a condition from which the individual in a normal state of nutrition promptly recovers, and the evidences of increased bone marrow activity are ordinarily shown in the peripheral blood by the appearance of augmented numbers of reticulocytes and occasionally of nucleated elements. At the same time, the red blood cell and hemoglobin values progressively increase toward normal. On the other hand, both animal experiments (31) and clinical observations make it clear that if the blood loss is sufficiently chronic, especially in the presence of dietary deficiency, hypochromic anemia without evidence of active blood regeneration will result when the available hematopoietic substances have been depleted to a certain level. The blood picture, under these circumstances, no longer shows evidence of increased bone marrow activity; the concentration of hemoglobin in the red cells becomes much reduced, and typical hypochromic anemia results.

McMaster and Haessler (32) showed that in rabbits in which anemia was induced by repeated bleeding, the blood was more quickly made up if the animals were given subcutaneous injections of hemoglobin. Miller and Rhoads (33) have recently shown that rabbits maintained at a hemoglobin level of approximately 50 per cent of normal by daily bleeding, will produce practically twice as much hemoglobin in a given length of time when the blood removed is laked and the hemoglobin reinjected as when the blood is discarded. Whipple

TABLE 5

Estimate of the number of times per month food of type specified was eaten by patients

CASE NUMBER	MEAT	MILK	EGGS	FISH	CEREALS	GREEN VEGETABLES	FRUIT	BUTTER
2	2	8	4	4	0	4	4	8
4	8	60	30	8	30	8	4	
5	8	8	8	12	8	12	30	
6	60	60*	30	2	0	12	60	30
7	8	30	30	2	30	60	30	30
8	3	1*	1	12	0	2	30	12
9	12	90	8	8	1	30	8	8
11	0	0	0	30	0	8	90	0
13	12	60	8	12	0	8	2	0
15	0	0	4	4	0	0	30	
16	0	30*	30	4	0	12	60	30
23	2	4	8	8	1	3	12	8
24	20	60*	60	20	1	30	60	30
25	2	60*	12	30	0	12	8	8
26	8	90	12	12	0	3	30	4
29								
30	4	30*	12	30	0	3	12	
32	0	0	8	8	0	30	30	
33	4	0	12	12	0	4	12	
34	0	0	0	30	0	12	90	0
35	8	30*	30	2	0	0	60	0
36	1	0	8	20	0	4	12	
37	20	30	30	8	0	12	12	
40	8	0	4	1	0	4	20	0
41	4	60	2	8	0	12	60	4
43	3	60	2	8	0	1	2	2
44	4	0	30	8	0	8	12	
45	0	20	1	8	0	2	4	
46	0	0	4	0	0	2	8	
48	1	4	4	30	0	8	8	4
49	4	30	0	8	0	3	30	0
50	4	20	4	4	0	2	2	0
51	8	30	4	8	1	4	20	4
52	3	30	12	20	0	3	30	0
53	8	0	8	20	4	4	30	
54	4	20	8	4	0	4	4	
56	12	30	0	1	0	60	30	0
57	8	30	4	0	0	8	8	4
58	6	30	30	12	12	30	30	30
59	12	30	4	16	4	30	20	0
61	12	30	2	24	2	60	40	2
62	12	0	0	8	0	12	2	30

TABLE 5—*Concluded*

CASE NUMBER	MEAT	MILK	EGGS	FISH	CEREALS	GREEN VEGETABLES	FRUIT	BUTTER
64	8	20	4	12	0	30	4	0
65	4	60*	30	30	0	12	90	0
66	12	60	8	16	3	8	24	4
67	12	4*	4	30	0	8	8	
68	0	30*	8	30	0	8	30	1
69	0	16*	12	30	0	12	6	0
71	20	60	0	8	4	30	30	0
72	4	4	8	30	0	8	8	
74	4	30	2	20	0	30	30	20
75	8	30	12	0	0	30	20	30
79	8	20	4	4	0	30	30	0
80	12	30	30	20	0	8	60	
81	0	0	0	4	0	0	4	
82	32	30	30	16	0	30	4	2

* Milk other than in coffee.

and Robscheit-Robbins (34) have demonstrated that in dogs with severe anemia from blood loss there is an 80 to 90 per cent utilization of hemoglobin given intravenously or intraperitoneally. The present observations demonstrate that human red blood cells also contain hematopoietic substances. Into each of 2 patients, cases 20 and 36, 30 cc. of washed, packed human red blood cells were injected intramuscularly daily for six days. The hematological data are shown in table 6. Although the results of the injection of these relatively small amounts of red blood cells, assuming even a 90 per cent utilization, could not be expected to be striking, it is interesting to note that in case 20 a gain of 1.97 red blood cells per cubic millimeter and 9 per cent of hemoglobin resulted within a period of sixteen days. In case 36 the gain of red blood cells and hemoglobin was insignificant, but a definite increase of reticulocytes appeared and reached a maximum of 12.6 per cent on the eighteenth day after the beginning of the injections. Similarly, in case 20 a reticulocyte peak of 6.8 per cent was attained on the twelfth day. No hematopoietic effects were observed from the injection of cell-free plasma. These results are considered to add to the evidence from other types of observations (32) (33) (34) that blood loss is important in the production of hypochromic anemia, not only as a result of the actual blood shed, but also as a result of the deprivation of the body of hematopoietic factors contained in the lost red blood cells. Whatever blood loss is then due to the hookworm is clearly a potential contributory factor to the development of the hypochromic anemia through both of these mechanisms.

2. The relation of factors other than the hookworm to the anemia. The work of many authors, especially that of Meulengracht (35), Witts (36), and Heath (37), suggests that hypochromic anemia in the temperate zone is due mainly to a lack of available iron in the body. The failure of the bone marrow to produce adequate amounts of hemoglobin and red blood cells is apparently at the bottom of the problem, and results directly or indirectly from an inability of the bone marrow to function normally in the absence of sufficient iron and probably of other hematopoietic substances. In hypochromic anemia, not associated with infection, the mechanisms by which the failure of hematopoiesis is brought about are various and may be combined in the same patient. The available iron in the body may be depleted by blood loss or the iron intake may be insufficient either because of dietary defect or because the absorption of iron from the intestinal tract is inadequate. In the latter case gastric anacidity (38) and conditions producing diarrhoea (39) probably play an important rôle. Although the ability of the hookworm to cause blood loss is clear, the failure of correlations of the degree of anemia with the number of parasites harbored by different individuals, clearly suggests the influence of additional factors. Furthermore, those communities in which hypochromic anemia associated with hookworm infestation is common, also furnish examples of similar types of anemia in individuals who have never had hookworm disease. Since, as has been demonstrated, the hypochromic anemia of hookworm disease has no morphological or physiological characteristics which distinguish it from hypochromic anemias without hookworm infestation, it is logical to look for the possible influence of factors common in producing other types of hypochromic anemia.

a. The relation of dietary defects to the anemia. Smillie (5) has suggested the importance of malnutrition in augmenting the anemia of hookworm disease in Brazil. Smillie and Augustine (23) thought

TABLE 6

Comparison of effects upon hematopoiesis of administration of various extracts of liver, of washed erythrocytes and of ferric ammonium citrate to patients upon basal diets without removal of hookworms

First periods. Daily administration of various substances as indicated below

	LIVER EXTRACT E-29 DERIVED FROM 340 GRAMS OF LIVER (CONCENTRATED AQUEOUS EXTRACT)											
	Case 1			Case 8			Case 26*			Case 64		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	3.91	35	1.6	2.53	32	5.2	1.07	10	4.4	3.57	44	5.2
2	4.02	34	1.8	2.46	35	4.0	1.16	9	3.0	3.67	42	4.0
4	3.78	40	1.4	2.66	34	5.4	1.01	9	2.6	3.03	41	5.8
6	3.68	38	3.0	2.86	40	*13.8*	1.13	13	*13.8*	3.60	42	*8.0*
8	3.41	37	*5.0*	3.14	42	12.6	1.34	18	9.8	3.54	44	5.0
10	4.06	42	2.8	3.30	43	10.6	1.31	19	7.4	3.65	43	6.0
12	4.00	39	3.4	3.47	50	7.6						
14				3.59	49	4.8						
16	3.86	43	1.0	4.02	58	5.0						
18												
20												
22	4.04	47	2.2									
24												
26												
28	4.05	53	3.4									
30												
32												
34	4.08	51										

	LIVER EXTRACT NO. 55, DERIVED FROM 400 GRAMS OF LIVER WITHOUT ADDED IRON (FRACTION INSOLUBLE IN 70 PER CENT ALCOHOL)								
	Case 22			Case 51			Case 36b		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	2.11	24	2.4	3.17	37	2.2	2.06	20	5.4
2	2.32	26	2.2	3.07	34	—	1.95	20	4.2
4	2.50	23	3.6	2.84	35	2.0	1.73	20	6.6
6	2.15	23	2.4	3.13	33	1.0	2.47	22	9.0
8	2.26	20	2.8	3.17	34	4.2	2.49	26	*13.4*
10	2.38	19	2.8	2.86	33	*5.2*	2.38	26	8.4
12				3.27	37	2.8			
14									
16									
18									
20									
22									
24									
26									
28									
30									
32									
34									

	LIVER EXTRACT NO. 343, N.N.R. DERIVED FROM 300 GRAMS OF LIVER (FRACTION SOLUBLE IN 70; INSOLUBLE IN 95 PER CENT ALCOHOL)					
	Case 27			Case 61		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	2.09	22	6.4	2.02	21	2.4
2	2.24	23	4.0	2.12	18	0.8
4	2.19	24	1.6	1.89	19	1.8
6	2.14	24	4.8	1.81	21	2.6
8	2.26	26	3.8	1.94	20	1.2
10	2.53	25	3.8	1.86	20	4.8
12	2.60	28	2.6			
14						
16						
18						
20						
22						
24						
26						
28						
30						
32						
34						

	WASHED ERYTHROCYTES FROM 30 CC. NORMAL BLOOD INTRAMUSCULARLY DURING FIRST 6 DAYS					
	Case 20			Case 36a**		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	2.26	30	2.8	1.41	17	3.0
2	2.97	26	2.0	1.43	16	2.4
4	3.27	28	2.6	1.63	15	3.6
6	3.49	29	2.6	1.42	15	2.8
8	2.86	30	5.0	1.21	15	3.4
10	3.28	31	6.6	1.08	13	5.0
12	4.11	35	*6.8*	1.09	14	10.0
14	4.22	36	3.6	1.33	14	8.8
16	4.23	39	5.2	1.38	16	8.8
18				1.99	22	*12.6*
20				1.54	20	4.6
22						
24						
26						
28						
30						
32						
34						

* Received extract from only 240 grams of liver daily.

** Periods preceding other observations shown above on same case (36b)

Second periods. Daily administration of various substances as indicated below

	FERRIC AMMONIUM CITRATE, 6 GRAMS						FERRIC AMMONIUM CITRATE, 4 GRAMS			FERRIC AMMONIUM CITRATE, 6 GRAMS			FERRIC AMMONIUM CITRATE, 2 GRAMS						FERRIC AMMONIUM CITRATE, 6 GRAMS			LIVER EXTRACT E-29 DERIVED FROM 340 GRAMS OF LIVER						FERRIC AMMONIUM CITRATE 50 MGM. INTRAMUSCULARLY FIRST 6 DAYS			FERRIC AMMONIUM CITRATE 100 MGM. INTRAMUSCULARLY FIRST 5 DAYS		
0	4.08	51		4.02	58	5.0	1.31	19	7.4	3.65	43	6.0	2.38	19	2.8	3.27	37	2.8	2.38	26	8.4	2.60	28	2.6	1.86	20	4.8	4.32	39	5.2	1.54	20	4.6
2				3.91	59	3.0	1.36	26	14.0	3.82	47	3.8	2.30	22	2.8	3.48	35	2.8	2.86	31	8.2	2.63	27	3.2	2.12	23	4.4	4.28	34	5.4	1.61	20	5.4
4				3.80	60	*6.0*	2.03	27	*17.0*	3.77	48	6.6	2.55	26	5.2	3.58	38	6.2	2.80	36	*14.0*	2.87	28	7.6	2.22	20	3.0	4.32	34	4.8	1.74	19	3.8
6				3.70	66	4.4	2.21	31	10.0	3.33	47	5.8	2.65	29	*10.0*	3.77	36	8.0	2.93	44	9.6	2.83	35	9.8	2.10	23	3.6	3.72	37	5.2	1.43	19	6.6
8	3.73	56		4.00	61	3.2	2.23	28	13.4	3.93	52	*8.4*	2.98	32	10.0	3.50	38	*8.2*	2.83	49	8.8	3.14	38	10.0	2.59	27	*6.8*	4.22	39	3.6	1.60	23	*7.0*
10				4.23	69	2.2	2.80	35	7.8	3.74	57	3.8	3.38	39	9.6	4.15	40	7.0	2.94	52	8.0	3.53	44	*11.0*	2.79	28	3.8	4.33	39	2.6	1.96	21	5.2
12							3.08	39	14.2				3.31	37	8.6	4.08	46	6.0	3.04	49	4.4	3.38	42	5.0	2.74	29	5.8	4.24	40	2.2	2.06	20	5.4
14	4.78	60					3.19	40	7.6				3.46	42	8.2	3.89	43	5.4	3.60	60	4.8	3.30	41	5.2	*2.98*	*34*	*3.8*				1.95	20	4.2
16							3.26	44	6.8				3.36	47	8.0	3.54	43	3.8	3.45	56	4.0	3.47	45	5.2	*Fe. Am. Citr. 6 gm.*								
18							3.21	46	4.4				3.67	52		3.47	43	4.0				3.61	45	8.4	3.52	40	*12.6*						
20							3.43	45	3.8				3.49	58	7.0	*3.61*	*44*	*4.6*				3.88	50	6.8	3.46	46	10.4						
22	3.63	57		4.43	72	1.0	3.51	48	4.8				3.61	57	6.4	*Fe. Am. Citr. 6 gm.*						3.83	49	5.2	3.39	47	4.8						
24							3.21	46	4.8				3.96	59	3.4	3.91	53	8.4	4.04	65	2.0	3.89	52	4.4	4.03	51	4.0						
26							3.58	51	2.0				3.91	58		4.06	58	*10.4*				3.92	52	3.6	4.14	55	3.6						
28	3.78	57											4.42	66	1.2	4.33	57	6.6				3.96	54	2.6	4.02	57	1.8						
30																4.14	60	6.0	4.41	73	1.2												
32																																	
34				4.74	75		4.60	67	1.4													3.33	63	2.2	3.89	65							
weeks																																	
6																			4.13	79		4.12	72		4.14	69	0.2						
8				3.81	72	2.4	4.41	63	0.6													4.88	78	1.0	4.60	70	1.4						
10				4.38	76		4.00	65	1.4													5.20	78	0.6									
Ova per cgm. stool....	Positive			357			494			118			Positive			250			130			286			101			4			130		

that with ample food there could be a heavy hookworm burden without anemia. Schapiro (6) noted differences between the worm burden and degree of anemia in the population of Panama, which he attributed to differences in diet. An investigation was made of the individual diets of 56 of the 83 patients presented in this report selected entirely at random. The method of taking the diet histories included questioning the patient as to his particular likes and dislikes in food, and whether his diet differed in any way from that of other members of his family. He was asked to describe an average daily menu. In addition, he was questioned as to the number of times per day, week or month that he ate specific foods common in Puerto Rican dietaries. Although the taking of a dietary history is admittedly a difficult procedure in which the quality of the patient's answers may be as im-

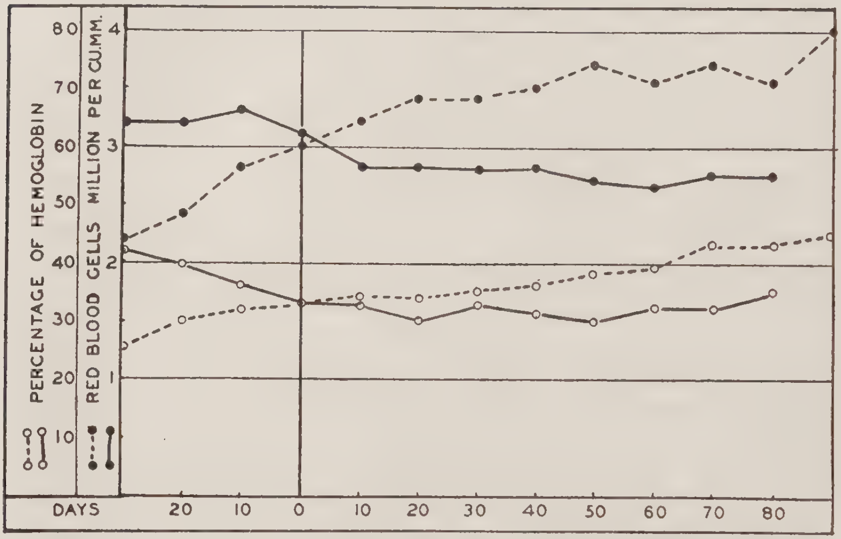

Fig. 6. Effect of the Removal of the Parasites upon Red Blood Cell and Hemoglobin Production in Patients Not Receiving Iron

The average values of the red blood cells of the 11 patients of groups A-I and A-II (solid dots and broken line), whose hookworms were removed, should be compared with the average values of the red blood cells of the 9 patients of groups B-I and B-II (solid dots and line), whose hookworm burden was not removed. The average hemoglobin values of the same groups of patients are shown in the curves with open dots. The differences in the trend of the curves represent differences induced by removal of the parasites. Note that the effect of removal of the parasites upon red blood cell production is greater than upon hemoglobin production.

portant as his quantitative statements in indicating the amount and type of food taken, the monotony of the fare of the Puerto Rican peasants aided the investigation by limiting the possibilities. In general, the diets were very similar and could well be described in the words of Suárez (40):

> The Porto Rican peasant breakfasts on coffee and a small loaf of pale white bread. His lunch consists of a large quantity of polished rice and kidney beans with scanty vegetables. His dinner is a stew of rice, chick-peas, pumpkin, and perhaps one or two tropical vegetables such as yautiá. Once or twice a week he has the great good fortune to eat some salted codfish. This diet has been taken without variation since childhood. Many of these individuals have never had meat, eggs, or milk.

In table 5 an attempt has been made to present in a roughly quantitative manner the individual consumption of those articles of diet which might be considered to be important from the standpoint of sources of complete proteins, vitamins, and mineral elements including iron. Thus, the table does not represent the chief sources of energy of the Puerto Rican diet mentioned by Suárez, but merely those foodstuffs considered to be of especial nutritional significance. The figures represent as nearly as could be determined the average number of times, without reference to quantity consumed, that the particular type of food mentioned was eaten during a period of one month. It is to be clearly understood that the figures are estimates and are necessarily of only approximate accuracy. From an inspection of the data, however, it is at once apparent that the consumption of meat, milk, eggs, whole grain cereals, and butter was extremely low in this group of patients. Milk was utilized mainly in small amounts in coffee unless indicated in the table by an asterisk. Fresh fish was rarely used, and the values in the table refer almost entirely to salt codfish. Green vegetables, such as lettuce, chard, string beans, chayote, okra, and other Puerto Rican products were largely employed in garnishing the main staples, rice and beans. In a land in which citrous fruits, pineapples, and bananas are plentiful, it is surprising to see how small the consumption of these articles appeared to be.

The explanation is largely an economic one, for the majority of the population affected by hookworm anemia is of the peasant class, serving as laborers on plantations for the raising of such "gold crops" as sugar, coffee, and tobacco. The concentration of the living quarters of the laborers makes small truck farming difficult, or it may even be expressly forbidden by the policy of the company. The wage scale is low, and the dependents are often numerous. Several of the patients in this group depended for their food entirely upon the charity of others. Poverty, local customs, and lack of refrigeration diminish to a minimum the consumption of meat and dairy products, and result in the widespread use of dry beans and peas, polished rice, and salt codfish in a land seemingly bountifully endowed by nature.

It has been clearly shown by many workers (35) (36) that iron in a variety of forms is of dominant importance in the therapy of hypochromic anemia. Since Heath, Strauss, and Castle (37) have shown that in hypochromic anemia, iron administered parenterally reappears more or less quantitatively in the resultant increased hemoglobin in the blood stream, the view that iron therapy acts more or less directly by eliminating an iron deficit in the body is highly credible. For this reason it is logical to suppose that a lack of iron in the diet may lead to a deficiency of hemoglobin in the red blood cells. According to the figures given by Sherman (41) the percentage iron content of the main articles of these Puerto Rican diets may be assumed to be somewhat as follows: dry kidney beans, 0.007; dry peas, 0.0057; white bread, 0.0009; fruit and tropical vegetables less than 0.001; codfish at least 0.0011. Lean meat and eggs each contain 0.003 per cent of iron, but the other articles itemized in table 5, such as milk, cereal, green vegetables, and butter, all contain less than 0.001 per cent of iron. It is thus apparent that, because of the addition of peas and beans, there exists in these diets a reasonably good source of iron when compared with the figures, for example, for lean meat and eggs. According to Robscheit-Robbins (42) certain foodstuffs, especially glandular organs, such as liver, kidney and chicken gizzard, and such foods as eggs, apricots, and, to a lesser extent, lean meat, are of importance in producing hemoglobin in the dog. It is clear that these articles were present to only a small degree in Puerto Rican diets.

Based on the available knowledge, the diets seem possibly to have contained in the peas and beans good sources of iron, although they lacked decidedly the food substances that by reason of other properties may be of metabolic importance in the production of hemoglobin. In the light of these facts, it is certainly not possible to arrive at any definite conclusion concerning the exact nature of the dietary defect related to the anemia. It is clear, however, that the supply of animal protein, minerals, and possibly of certain vitamins was far from optimal in these diets. Cook (43) states that 50 per cent of the energy value of the foods imported in Puerto Rico is represented by rice and beans. He showed that prepared rice and beans, as served in a Puerto Rican restaurant, were inadequate for growth in rats unless supplemented with cod liver oil. Certain common Puerto Rican vegetables, such as plátano and yautiá, were of some assistance in promoting growth when added to the rice and beans. Cook and Rivera (44) found the calcium intake to be low in the Puerto Rican diets. Although the exact relation of dietary constituents to anemia is still uncertain, the clinical impression is that many cases of hypochromic anemia, not associated with hookworm infestation, are observed in the temperate zone, in which diets far less impressively defective than these appear to be of etiological significance. On the other hand, the appearance of anemia in certain individuals is incomprehensible except on the assumption that changes in the assimilative capacity of the intestinal tract render such individuals different from other members of the community. In the light of the observations of McCarrison (45) and others, it is probable that defective nutrition also exercises an effect indirectly, by promoting changes in the function of the gastrointestinal tract. Such changes may then secondarily lead to difficulty with the assimilation of elements directly essential to hematopoiesis.

b. The relation of gastrointestinal pathology to the anemia. The concept of deficiency disease "conditioned" by gastrointestinal defects has been elaborated, using as an illustration Addisonian pernicious anemia (46). The apparent relation of altered gastrointestinal function to hypochromic anemia has been emphasized by the work of Mettier and Minot (38), Keefer (39) (47), and others (48) (49). Dysphagia, gastric anacidity, and diarrhoea have been shown to occur in many cases of hypochromic anemia and to have a probable etiological relationship by causing the individual to limit or modify his diet or by preventing the proper assimilation of the elements necessary for hematopoiesis. Ashford and his coworkers (8) stated that in hookworm disease the tongue was at times partially denuded of epithelium and was raw and red. They noted that a severe stomatitis was sometimes present, even involving the buccal mucous membranes. Pain and tenderness in the epigastrium were found to be the most constant and clearly marked of any of the symptoms referable to the digestive tract. General abdominal tenderness was occasionally present. In their experience constipation was the rule, but in the advanced stages of the disease this alternated with diarrhoea.

The factors of glossitis, gastric anacidity, and diarrhoea were accordingly given special attention in the analysis of the causes of this type of hypochromic anemia. In table 1 are shown the results of these investigations. Of 47 patients adequately studied, 8 either gave a history of or showed lingual changes. This is of importance because of the common association of loss of the papillae of the tongue in hypochromic anemia and in other deficiency diseases (48) (50). The gastric response to the administration of 50 cc. of 7 per cent alcohol and to the subcutaneous injection of 0.5 mgm. of histamine phosphate was determined in 54 patients, usually once, either before treatment or in the early stages of effective therapy. The pepsin content of the material secreted in response to histamine was estimated by the use of Mett's tubes in samples of gastric contents brought, if necessary, to pH 2 with hydrochloric acid. Of the 54 patients examined, free hydrochloric acid was secreted in response to the alcohol by 30, and only after the injection of histamine by 11; in 13 patients there was no secretion of free hydrochloric acid. Peptic activity was observed in all but 7 patients, of whom only one was able to secrete hydrochloric acid. That gastric anacidity was found in only 24 per cent of the patients with hookworm disease and anemia indicates that it is by no means as common as in idiopathic hypochromic anemia (20) (51). However, since Mettier and Minot (38) were able to demonstrate greater effects with iron salts administered to patients with idiopathic hypochromic anemia when the upper intestinal contents were kept relatively acid than when maintained near the neutral point, the etiological significance of achlorhydria in relation to the absorption of iron is probably of potential significance. In the etiology of the hypochromic anemia of hookworm disease, however, the much lower incidence of gastric anacidity correspondingly diminishes the importance of this factor.

Since the patients with anemia unassociated with hookworm infection were observed in a community in which sprue is common, it is possible that the defective dietary background considered to be an etiological factor in sprue (52) (53) (54) has an influence upon the patient with hypochromic anemia. As was stated above, the distinction between cases of sprue with hypochromic anemia and cases of hookworm disease with lingual and intestinal symptoms was necessarily arbitrary in some instances. Indeed, of the 47 patients selected, 8 had a history of or showed signs of glossitis and 24 had had moderately severe diarrhoea on certain occasions preceding the present illness. The fact that transient diarrhoea is a common occurrence in people in the tropics who are apparently in good health otherwise, should not set aside its potential significance as a mechanism leading to the faulty absorption of hematopoietic factors in the light of the observations of Keefer (39) (47) and others. In studying patients with sprue, in certain instances the effectiveness of orally administered liver extract was found to diminish as time went on, although its activity by parenteral injection remained good (55) (56). This was regarded as evidence of a progressively decreased absorption of the orally administered active principle. By analogy it is perhaps reasonable to assume that the intestinal tract of certain individuals who live on defective diets and who harbor hookworms may become less permeable to iron. Such a supposition is clearly necessary in comprehending the etiology of certain cases of hypochromic anemia not associated with hookworm infection. Here again the oral dosage of iron salts necessary to produce a given hematopoietic effect varies from patient to patient, as does the dosage of liver extract in different patients with pernicious anemia or sprue. Rotter (57) described the occurrence of morphological change in the small intestine in hookworm disease. He stated that the submucosa was thickened, with hyaline changes of the connective tissue and deposit of hemosiderin. The mucosa may be from 3 to 5 times as thick as the normal, with areas of atrophied mucosa containing very few glands. At any rate, the association of changes in the gastrointestinal tract with other types of hypochromic anemia is so common that in the face of the available evidence, such changes must surely be considered as a factor in the etiology of the hypochromic anemia associated with hookworm infestation.

IV. OBSERVATIONS CONCERNING THE TREATMENT OF THE ANEMIA

Although a few reports on the effect on administering hematopoietic substances upon the anemia associated with hookworm disease existed in the literature at the time that these studies were undertaken, the attention of those engaged in treating patients with hookworm disease was largely taken up with the elimination of the parasite. A careful analysis of the relative effects of the removal of the parasites, and of therapy with improved diets, with iron and with certain liver extracts has not to our knowledge, however, previously been carried out under controlled conditions in hospitalized patients as well as in field experiments. Since the publication of the preliminary report of this paper (11), Suárez (58) and Cruz (59) have confirmed certain of the results described here in detail.

A. The effect of removing the hookworms. The effectiveness of removing the hookworm burden in bringing about relief of the anemia has not always been uniform in the experience of others (8) (19) (60), although the elimination of the parasites has generally been considered necessary to a cure. Ashford, King, and Igaravídez (8) concluded from their observations in Puerto Rico that the elimination of the hookworms would bring about rapid relief of the anemia in the majority of instances. The present observations present a striking contrast to their conclusions since the effect of removal of the parasites without other therapy was, at best, slight. The essentially negative effect of removing the parasites from 12 patients who were maintained under basal conditions in the hospital has been described in detail above (see table 2 and figure 3). Below are described the confirmatory results of similar observations in the field, which are summarized in table 9 and figures 5 and 6.

B. The effect of iron salts. Although Ashford, King, and Igaravídez (8) did not consider therapy with iron of any particular importance, compared to elimination of the hookworms, in the relief of the anemia, a careful scrutiny of the protocols of their observations suggests that the iron administered in many instances was of importance in bringing about the rapid improvement observed. This interpretation finds support in the observations of others. Boycott (60) observed improvement of the blood picture in hookworm disease with iron and arsenic therapy. Day and Ferguson (19) showed striking improvement on giving iron. Kobayashi (61) and Yang and Keefer (62) demonstrated the positive effect of the administration of iron upon hematopoiesis in a few patients without removing the parasites. As described above, the daily administration of 6 grams of ferric ammonium citrate *without removal of the parasites* was attended in our experience by rapid relief of the anemia. The data for such observations upon 27 patients are presented in tables 3 and 4. In table 4 are also shown the results of similar therapy upon the hemoglobin values of 6 patients whose hookworm burden had previously been removed. The average increases of hemoglobin in the two series during the first thirty days were 25 and 31 per cent respectively. These values compare favorably with the results of iron therapy in other types of hypochromic anemia; and were confirmed by the studies in the field described below. In the group of 6 patients whose hookworm burden was not removed the average gain of hemoglobin in thirty days was 38 per cent, whereas in the group of 5 patients whose hookworms were eliminated the gain was 34 per cent in thirty days.

Thus, the lesson is clear, that the removal of the hookworm burden has in itself no especial effect upon hematopoiesis, whereas the administration of iron salts, irrespective of the presence of the parasite, at once brings about rapid hemoglobin regeneration. Moreover, the increase in hemoglobin values is coincident with remarkable clinical improvement. Marked subjective improvement was sometimes experienced within two or three days. Thereafter the lassitude rapidly diminished, the color and strength improved, the appetite increased, edema disappeared, and the circulatory signs of anemia were progressively relieved. Moreover, the gastrointestinal symptoms of the disease, sometimes ascribed to the irritating effect of the parasites, were progressively relieved as the blood values rose, whether the hookworms were removed or not. Improvement of the glossitis, as in idiopathic hypochromic anemia, was also observed. This is the more remarkable since the only change in therapy had been the addition of iron salts to the basal or native diets. On the contrary, the effect of simply removing the parasites improved the clinical condition of the patients no more than it did their blood values. They remained pale, weak, and apathetic.

It will be noted that in many instances the hemoglobin values in the tables had not reached normal values by the end of the periods of iron administration. This is due in part to the fact that the data do not extend beyond the termination of the particular type of observation under consideration, or that the patients left the clinic or hospital. For obvious reasons the effects of various procedures subsequently carried out on certain patients could not be shown in detail in the tables. However, although the patients regarded themselves as entirely well, the gain in hemoglobin, after a value of about 70 per cent was reached as a result of the administration of iron, was often slow as in other types of hypochromic anemia. The effect of removing the parasites or of giving in addition aqueous extract of liver was, therefore, tried in certain patients whose hemoglobin values seemed to be making slow progress. Neither of these procedures could be demonstrated to have any particular effect, and it became evident that time was necessary in many instances to complete the results of iron therapy. Furthermore, if permanent changes in the absorptive capacity of the gastrointestinal tract have occurred, as apparently exist in other types of hypochromic anemia, it is probably necessary in certain patients to continue a maintenance dose of iron even after the blood values are normal (63).

C. The effect of various extracts of liver. Cohn, Minot, and their collaborators (64) developed the first fractions of liver for the treatment of pernicious anemia. Based on their methods the processes now employed consist essentially in the following steps: Beef or pig liver is finely minced and extracted with water. Upon acidification to pH 5 and heating to 80°C. a copious precipitate results. The remaining liquid is then concentrated in vacuo to a syrupy consistency. This fraction is effective in the treatment of pernicious anemia and was successfully used by Suárez (9) in the treatment of the anemia of hookworm disease. When the syrup is poured into alcohol in such amounts that a 70 per cent concentration of alcohol by volume is obtained, a second precipitate results. This fraction has been found effective by Whipple and his coworkers (65) in the dietary-blood loss anemia of dogs. A considerable portion of the material soluble in 70 per cent alcohol may be precipitated by the addition of absolute alcohol until a concentration of 95 per cent by volume is reached. The dried precipitate, which is the original "fraction G" of Cohn, is highly effective in pernicious anemia but has little or no effect in hypochromic anemia (13) (63).

Accordingly certain commercial preparations of these fractions of liver were administered to suitable patients under the usual controlled conditions in the hospital. The effect of the simple aqueous concentrate[2] previously used by Suárez (9) was evaluated in dosages known to be effective in pernicious anemia. Observations were made on 15 patients. In table 6 are shown in detail the data concerning red blood cells, hemoglobin and reticulocytes during observations on 6 patients, cases 1, 8, 26, 27, 61, and 64. No anthelmintic treatment was given. During periods of several days the amount of Liver Extract E-29 derived from 340 grams of liver was given daily to each patient, except to case 26 (a boy of 9 years) who received the amount derived from only 240 grams. With the exception of case 64, there was in each patient an increase of reticulocytes followed by an increase of hemoglobin. In cases 8, 26 and 64, in immediately subsequent periods following the discontinuance of Liver Extract E-29, the daily administration of ferric ammonium citrate produced second reticulocyte peaks. In table 7 are summarized the data upon hemoglobin production in 15 patients treated daily with the amount of Liver Extract E-29 derived from 340 grams of liver. These data were derived from observations of the 6 patients just cited and 9 others observed as out-patients. The average increase of hemoglobin for the group in thirty days was only 13 per cent compared with 25 per cent in a group of 27 other patients receiving 6 grams of ferric ammonium citrate daily (see table 4). These observations confirm the activity of Liver Extract E-29 in this type of anemia noted by Suárez, but indicate the greater effectiveness of the iron salt, at least in the dosages employed. The relative effectiveness of these substances thus resembles the results in other types of hypochromic anemia.

The fraction of liver which is insoluble in 70 per cent alcohol is relatively ineffective in the treatment of pernicious anemia. It corresponds, however, to the fraction shown by Whipple and his associates (65) to have hemoglobin-producing ability in anemic dogs. This substance is commercially available in dry form as Liver Extract No. 55, except that in this particular preparation approximately 0.5 gram of ferric ammonium citrate has been added to the amount of extract derived from 100 grams of liver. Without the iron, this material[3] was administered daily to 7 patients in doses of 12 grams, representing the extract derived from 400 grams of liver. In 3 patients there was no significant effect upon hematopoiesis during periods of ten days as illustrated by the data from cases 22 and 51 shown in table 6. In the other 4 patients of this series, there was a moderate effect upon hematopoiesis from feeding this liver fraction (see case 36). In all instances there was a second increase of reticulocytes when the patients were given daily 2 grams of ferric ammo-

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[2] This fraction is commercially available as Liver Extract E-29, kindly supplied by Valentine's Meat Juice Company.

TABLE 7

Effect upon hemoglobin production of daily administration of liver extract E-29 derived from 340 grams of liver

Hookworms not removed

CASE NUMBER	HEMOGLOBIN PERCENTAGES AT 10-DAY INTERVALS						
	0 days	10 days	20 days	30 days	40 days	50 days	60 days
1	34	40	46	52			
2	15	18	30				
8	35	46					
15	28	34	38	45	47	51	
19	22	32	40	44			
27	28	41	45				
31	31	41	40	42	42		
44	23	24	28	33			
45	28	35	38	40			
50	38	39	44	50	51		
54	36	40	40	42	46	49	53
61	20	27					
64	42	45					
75	34	39					
Averages....	30	36	39	43	47	50	53

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[3] Kindly supplied without added iron by Eli Lilly and Company.

nium citrate, which is the amount of that iron salt added to each 12 grams of the liver fraction in the preparation of Liver Extract No. 55. Thus, the administration of the fraction of liver precipitated by 70 per cent alcohol had relatively slight hematopoietic effects in contrast to the action of the relatively small dose of ferric ammonium citrate. The effect of 2 grams of ferric ammonium citrate upon red blood cell and hemoglobin values was striking but less than that of 6 grams of the same preparation. (See case 51.)

In considering the activity of these liver extracts, the fact that a certain amount of iron was naturally present is possibly of significance. The daily dose of Liver Extract E-29, derived from 340 grams of liver, probably contained between 8 and 25 mgm. of iron. This would correspond to 50 to 156 mgm. of ferric ammonium citrate. The smaller dose would scarcely be of significance, but the effect of the larger amount might be detectable. In the daily dose of Liver Extract No. 55, derived from 400 grams of liver, about 34 mgm. of iron were present according to one analysis. This would correspond to about 210 mgm. of ferric ammonium citrate, which would probably have a slight effect and might, in some part, account for the activity of the material when administered even without the iron added in its commercial preparation.

The fraction of liver which is soluble in 70 per cent alcohol but insoluble in 95 per cent alcohol by volume, is potent in pernicious anemia in daily doses of the amount derived from 300 grams of liver. Liver Extract No. 343, N. N. R., represents a commercial preparation of this fraction. The data for red blood cell, hemoglobin and reticulocyte values from observations on cases 27 and 61 are shown in the first periods of table 6. During periods of ten days the daily oral administration of the extract[4] derived from 300 grams of liver was without detectable effect in these and in two other patients. In case 27 the reticulocytes were originally elevated, but did not increase during the administration of this liver extract, as occurred immediately thereafter in both cases 27 and 61 with the administration of Liver Extract E-29. The daily intramuscular injection of the amount of Liver Extract No. 343, N. N. R., derived from 10 grams of liver has the

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[4] Kindly supplied by Eli Lilly and Company.

TABLE 8

Comparison of effects upon hematopoiesis of addition of meat and milk and of ferric ammonium citrate to basal diet without removal of hookworms

	CASE 29			CASE 35			CASE 53			CASE 58			CASE 62			CASE 65			CASE 82			CASE 83		
	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes	Red blood cells	Hemoglobin	Reticulocytes
First periods. Daily administration of 300 grams of meat and 1500 cc. of milk																								
days	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*	*millions*	*per cent*	*per cent*
0	2.91	25	2.6	3.28	47	2.6	1.81	31	0.8	1.94	24	6.0	2.14	32	3.0	1.73	19	2.6	2.88	37	3.6	3.71	46	1.8
2	2.81	28	4.8	3.11	43	2.4	2.03	28	2.8	2.30	28	10.8				1.46	19	2.4	2.70	37	3.4	3.60	47	1.8
4	2.92	28	3.8	3.59	47	4.0	2.40	28	2.8	2.33	24	5.4	1.81	26	4.6	1.50	20	4.0	3.04	42	3.6	3.67	50	3.2
6	2.75	28	5.8	3.43	46	2.2	2.08	25	3.0	2.25	25	5.2	1.79	29	4.2				3.00	36	3.2	3.99	53	3.0
8	3.10	32	3.6	3.40	44	2.4	2.02	21	2.0	2.20	21	6.4	1.82	27	5.4	1.71	19	3.0	2.68	36	3.8	3.92	54	2.2
10	3.34	37	3.8	3.39	44	2.4	1.76	20	4.4	2.08	22	3.8	1.59	26	4.8	1.38	18	3.4	3.24	37	2.4	3.98	51	2.8
12	3.33	36	2.8	3.50	48	1.8	1.89	23	4.0	2.11	23	5.0	1.57	25	4.2	1.58	20	2.8	2.89	37	3.2	3.73	45	2.6
14	2.81	30	3.4				1.86	22	4.6	2.34	26	4.2	1.58	24	4.2	1.56	20	4.6	3.11	39	3.6	3.99	53	2.2
16	2.40	28	4.6	3.38	42	1.6	1.93	21	4.6	—	—	—	1.46	23	4.8	—	—	—	2.67	36	2.8	4.03	50	2.6
18	—	—	—	3.39	41	1.8	—	—	—				1.46	22	6.2				2.76	38	2.2	3.76	48	1.8
Second periods. Daily administration of 6 grams of ferric ammonium citrate																								
0	2.40	28	4.6	3.39	41	1.8	1.93	21	4.6	2.34	26	4.2	1.46	22	6.2	1.56	20	4.6	2.76	38	2.2	3.76	48	1.8
2	2.48	25	6.6	3.56	44	4.0	1.93	21	8.6	2.30	26	7.4	1.39	22	3.0	1.98	21	4.6	2.86	38	1.4	3.92	54	1.8
4	2.71	30	6.2	3.91	50	*6.8*	1.85	23	12.0	2.58	29	15.0	1.45	24	3.0	2.12	23	10.2	3.05	41	3.0	4.10	51	1.8
6	2.73	32	*7.4*	3.97	57	3.4	2.21	33	*15.8*	2.67	37	*16.0*	1.87	30	3.8	2.07	28	*12.0*	3.20	43	*4.0*	4.24	57	2.6
8	2.91	37	6.6	4.36	59	3.2	2.54	34	13.8	3.14	40	12.8	2.10	37	8.6	2.09	29	5.6	3.60	50	3.4	4.60	60	1.2
10	2.82	41	4.6	4.59	61	4.8	2.30	38	9.8	3.25	44	6.6	2.05	35	*10.2*	2.25	31	5.2	4.15	49	2.2	4.32	59	1.0

12	2.70	46	3.8	4.99	63	2.6	1.98	44	10.2	3.48	52	8.2				2.88	38	4.4	4.10	51	1.8			
14	3.05	44	3.4	4.70	70	4.0	2.76	43	7.4				1.94	40	6.2									
16	3.14	42	1.2				3.15	47	7.0				2.00	41	6.0				4.20	56	1.8	4.42	62	1.2
18	3.54	49	2.0				2.82	46	5.8							3.04	47	6.0						
20	3.69	51	1.2				3.41	52	7.0	3.61	63	3.0							4.00	53				
22							3.82	58	3.0															
24																								
26							3.36	50	1.6				2.88	48	2.0									
28				4.79	70	0.6				4.03	66								4.05	58	2.4			
30																								
32																								
34							3.92	59		3.93	67	0.6	3.11	51	2.8									
Ova per cgm. stool....	158			237			931 (2,231 worms)*			113 (155 worms)*			607 (1,424 worms)*			601			124			Positive		

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* Number of worms subsequently removed.

hematopoietic effect in pernicious anemia of the daily oral administration of the same extract derived from at least 300 to 400 grams of liver. This extract[5] injected daily in amounts derived from 10 grams of liver had no effect on hematopoiesis in 8 patients, as illustrated by the data from the first periods of cases 5, 9 and 11, shown in table 3. The negative effect of this material in the hypochromic anemia of hookworm disease thus corresponds with the results in other types of hypochromic anemia.

D. The effect of improved diet. The deficient quality of the diet of the majority of these patients has been discussed above. It therefore seemed logical to evaluate its etiological and therapeutic implications by observing the effect upon the anemia of improving the diet. The basal diet in the hospital of 8 patients was accordingly improved by the daily addition of 1500 cc. of milk and 300 grams of lean beef. It was felt that these two substances would yield an excellent supply of animal protein, would provide certain vitamins, and serve as sources of iron and calcium. In table 8 are shown the detailed results of these dietary additions upon hematopoiesis. No treatment for the elimination of the parasites was given until after the completion of the observations. It will be seen that within periods of time varying from fourteen to eighteen days, no significant effect upon reticulocyte production, hemoglobin or red blood cell values was produced. Case 58 was apparently running an elevated reticulocyte count throughout the period of observation. Although in a few instances there were gradual increases of reticulocytes of slight degree, the absence of any significant effect produced by the improved diets is clearly brought out, upon the return to the basal diet, by the positive effect of the administration of ferric ammonium citrate in daily doses of 6 grams. In every instance, rises of red blood cell and hemoglobin values resulted, associated with increases of reticulocytes. The negative effect of the administration of the improved diet, contrasted with the positive effect of iron upon reticulocyte and hemoglobin production in cases 53 and 62, is graphically presented in figure 4.

V. OBSERVATIONS IN THE FIELD

The results in hospitalized patients of these observations upon the relative therapeutic efficacy of removal of the hookworms, of improved diets, and of iron therapy were confirmed in general by the field observations in Cidra on a group of 32 patients with initial hemoglobin values of less than 50 per cent of normal, and hookworm ova in the stools. These observations are being reported elsewhere in detail (12). The patients were largely employees of a local company, and lived in small houses scattered over an area of several miles. Men, women and adolescent children were selected at random, if found to be sufficiently anemic. Constant supervision was exercised to see that the medicine and food dispensed were actually taken by the proper individuals in the prescribed amounts.

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[5] Kindly supplied by Eli Lilly and Company.

The 32 patients were divided arbitrarily into two main groups, A and B, of 16 patients each. Those in group A were treated three times with carbon tetrachloride and oil of chenopodium followed by a saline purge before the beginning of the experimental period. By examination of the stools the elimination of the parasites was assured and maintained by re-treatment at approximately monthly intervals. In a few of these patients, despite three or more treatments, a few ova were constantly found in the stools. The patients of group B received no eliminative treatment, and the presence of a persistent hookworm infection was confirmed by examination of the stools for ova at frequent intervals. Each of the two main groups, A and B, was divided into three subgroups, I, II and III respectively. The patients in groups A-I and B-I had no change made in their diets during the whole course of the observations. The patients in groups A-II and B-II with the beginning of the experimental period were given daily 300 grams of lean meat and 1500 cc. of milk in addition to their usual diet. They were also encouraged to eat more fruit and leafy vegetables. The patients in groups A-III and B-III received no change in diet, but were given daily by mouth 6 grams of ferric ammonium citrate in the form of a 50 per cent aqueous solution. The blood studies consisted of capillary red blood cell and hemoglobin determinations, made at intervals of approximately a week during both the control and the experimental periods. The hemoglobin readings were invariably performed with the same Sahli instrument by the same observer.

For convenience in presenting the results of these observations, the hemoglobin determinations made upon each patient were plotted

TABLE 9

Comparison of effects upon hemoglobin production of removal of hookworms, of improved diet, and of administration of ferric ammonium citrate, in field observations at Cidra

Figures in table are hemoglobin percentages

GROUP NUMBER	CASE NUMBER	DAYS BEFORE			THERAPY	DAYS AFTER BEGINNING OF EXPERIMENTAL PERIODS									
		30	20	10		0	10	20	30	40	50	60	70	80	90
Group A. Hookworms removed															
A I	1			47	Diet not changed	50	52	46	49	57	56	52	54	57	56
	2		21	27		30	33	37	38	39	42	45	48	47	58
	3		34	30		28	31	34	36	32	30	28	45	41	
	4		39	40		40	41	40	42	41	42	48	52	53	48
	5			40		42	41	40	41	42	45	42			
	6		31	33		36	34	41	33	32					
	Averages		31	36		38	39	40	40	41	43	43	50	50	54
A II	7			30	300 grams meat, 1500 cc. milk added daily	31	35	37	41	43	41	44	49	51	48
	8		30	33		34	34	32	31	35	36	42	50	46	46
	9	34	34	35		31	31	29	28	28	40	38	36	29	32
	10	28	29	28		28	28	27	26	25	23	21	19	21	22
	11	12	9	14		18	17	19	21	25	26	29	24	28	30
	Averages	25	28	28		28	29	28	29	31	33	35	36	35	36
A III	12			26	Diet not changed; 6 grams ferric ammonium citrate daily	25	37	58	72	77	75	75			
	13			33		36	46	54	78						
	14	36	36	37		37	62	65	66	69	87	80	80		
	15	21	24	26		28	36	40	44	53	72	72	81		
	16			22		29	42	51	70	70	71	71	72		
	Averages	29	30	29		31	45	54	66	67	76	75	78		
Group B. Hookworms not removed															
B I	17				Diet not changed	38	36	24	22	25	25	25			
	18					28	20	19	19	22	20	17	24		
	19					30	31	28	24	37	32	33	31	27	
	Averages					32	29	24	22	28	26	25	28	27	
B II	20		39	35	300 grams meat, 1500 cc. milk added daily	36	40	38	40	46	46	46	46	48	48
	21	43	43	43		43	39	49	48	45	46	44	48	42	48
	22	44	43	43		43	46	40	32	36	36	31	32	34	31
	23	38	34	31		28	30	35	33	30	28	27	26	29	26
	24			29		23	25	18	13	11	8				
	25		31	30		31	35	35	38	33	33	26	25	24	20
	Averages	42	40	36		34	36	36	34	34	33	35	35	37	35

TABLE 9—*Concluded*

GROUP NUMBER	CASE NUMBER	DAYS BEFORE			THERAPY	DAYS AFTER BEGINNING OF EXPERIMENTAL PERIODS									
		30	20	10		0	10	20	30	40	50	60	70	80	90
Group B. Hookworms not removed—*Concluded*															
B III	26	19	18	18	Diet not changed; 6 grams ferric ammonium citrate daily	20	52	59	80	66	68	76	81		
	27		29	30		29	51	61	73	64	69	77	78		
	28		25	26		29	40	58	66	72	72	69			
	29					39	44	59	68	71	73	78	82		
	30	28	29	30		32	43	60	64	66	68	83	83	84	87
	31	43	38	35		36	57	62	70	73	74				
	32	34	36	37		38	57	70	68	67	73	75			
	Averages	31	29	29		32	49	61	70	68	71	76	81	84	87

against days, and from the curve so constructed the values at ten-day intervals were defined and are presented in table 9. From these data the average hemoglobin values for each group were calculated for ten-day intervals, and the results are plotted in figure 5. The average hemoglobin percentage of the 6 patients of group A-I, who had the hookworms removed but received no other therapy, shows a gradual upward trend, but in one hundred ten days the hemoglobin gain was only about 20 per cent. The average hemoglobin percentage of the 3 patients of group B-I, who received no treatment of any kind, decreased 5 per cent in a period of eighty days. The average hemoglobin percentage of the 5 patients of group A-II, who received the improved diet after having the parasites eliminated, showed an increase of 8 per cent in ninety days. The average hemoglobin percentage of the 6 patients of group B-II, who received the improved diet without removal of the parasites, was a few percent less at the end of one hundred twenty days. These results are then consistent with the assumption that by the removal of the parasites a constant source of blood loss was abolished, which resulted in the ability of the patients to gain hemoglobin very slowly, in contrast to the slow descent of the hemoglobin values of the patients still harboring parasites. They also confirm entirely the facts observed under controlled conditions in the hospital, that the removal of the parasites alone results in little improvement. The improved diet apparently was unable significantly to influence the rate of hemoglobin formation as already demonstrated by the observations on other patients in the hospital.

The data for the red blood cell values of individual patients were treated in a fashion similar to that applied to the data on hemoglobin and are shown in table 10. The removal of the parasites without other therapy permitted within thirty days moderate increases of erythrocytes in contrast to the insignificant effect upon hemoglobin formation. During periods as long as ninety days, somewhat greater increases took place. During one hundred ten days the average increases of red blood cells in groups A-I and A-II were respectively 1.6 and 2.0 million per cubic millimeter. In the corresponding groups in which the parasites were not removed, there were respectively decreases of 0.7 million in eighty days in group B-I and 0.4 million per cubic millimeter in one hundred ten days in group B-II. The averages of the combined red blood cell values of the 11 patients of groups A-I and A-II, and of the 9 patients of groups B-I and B-II, are plotted in figure 6. The average hemoglobin values of these two groups of patients are also plotted in the same figure. These data are thus derived from all the patients not receiving iron; and the differences between the two groups represent the effectiveness of removing the hookworm burden. In one hundred ten days the average increases of red blood cells and of hemoglobin of the 11 patients whose hookworm burden had been removed were respectively 1.8 million per cubic millimeter and 20 per cent. In one hundred days there were decreases of the average red blood cell and hemoglobin values of the 9 patients whose hookworm burden had not been eliminated, of 0.55 million per cubic millimeter and of 7 per cent, respectively. It is thus clear that the effect of removing the parasites is greater upon red blood cell than upon hemoglobin production, with a resultant lowering of the color index. This implies that the available hemoglobin has been redistributed among a larger number of corpuscles.

However, it was only when iron was given that both hemoglobin and red blood cell values were rapidly augmented, as shown by the data from the patients of groups A-III and B-III, given in tables 9 and 10. Under these circumstances, the presence of the parasites apparently had no detectable effect upon the production of either red blood cells or hemoglobin. Only by the use of iron was the oxygen-

TABLE 10

Comparison of effects upon red blood cell production of removal of hookworms, of improved diet, and of administration of ferric ammonium citrate, in field observations at Cidra

Figures in table are red blood cells in millions per cubic millimeter

GROUP NUMBER	CASE NUMBER	DAYS BEFORE			THERAPY	DAYS AFTER BEGINNING OF EXPERIMENTAL PERIODS									
		30	20	10		0	10	20	30	40	50	60	70	80	90
					Group A. Hookworms removed										
A I	1				Diet not changed	3.8	4.1	4.4	4.5	4.5	4.5	4.3	4.1	4.0	4.3
	2		1.9	2.4		2.9	3.4	3.7	3.5	3.5	3.5	4.1	4.8	4.4	4.3
	3		1.8	1.9		2.1	2.7	2.5	3.3	3.6	3.5	3.4	3.5	3.2	2.9
	4	2.2	2.7	3.2		3.7	3.8	3.6	3.8	4.1	4.4	4.1	3.9	3.7	3.5
	5			3.2		2.9	3.1	3.3	3.4	3.6	4.2	3.2			
	6		2.6	2.8		3.0	3.5	3.2	3.1	3.8					
	Averages	2.2	2.3	2.7		3.1	3.4	3.5	3.6	3.8	4.2	3.8	4.1	3.8	3.8
A II	7			3.4	300 grams meat, 1500 cc. milk added daily	3.8	3.9	3.5	3.8	3.6	3.2	3.8	4.1	4.0	4.6
	8		3.0	3.1		3.4	3.6	3.8	3.7	3.8	4.2	3.7	4.3	4.3	4.8
	9	3.0	3.5	3.9		3.0	2.6	3.8	3.4	3.3	3.7	3.5	3.4	2.5	3.2
	10	2.3	2.3	2.3		2.4	3.4	3.1	2.9	2.7	2.4	2.2	2.1	2.2	
	11	1.3	1.3	1.3		1.6	1.7	1.8	1.9	2.1	2.2	2.9	2.6	2.7	
	Averages	2.2	2.5	2.8		2.8	3.0	3.2	3.1	3.1	3.1	3.2	3.3	3.1	4.2
A III	12		1.7	1.7	Diet not changed; 6 grams ferric ammonium citrate daily	2.2	2.6	2.9	3.3	4.0	4.1	4.2	4.0		
	13			3.5		3.3	3.8	4.0	3.3						
	14	3.5	3.6	3.6		3.8	4.3	4.5	4.6	4.7	4.6	4.5	4.2	4.0	
	15	2.6	2.7	2.9		3.1	3.0	3.4	3.6	3.8	4.2	3.5	4.3		
	16			2.7		3.0	3.8	3.8	3.9	4.6	4.3	3.8	4.4		
	Averages	3.1	2.7	2.9		3.1	3.5	3.7	3.7	4.3	4.3	4.0	4.2	4.0	
					Group B. Hookworms not removed										
B I	17				Diet not changed	3.0	2.6	2.5	2.9	3.5	3.3	3.2			
	18					2.4	1.5	1.4	1.6	1.6	1.3	1.1	2.1		
	19					3.5	3.0	3.2	3.2	2.8	2.7	2.0	2.0	2.3	
	Averages					3.0	2.4	2.4	2.6	2.6	2.4	2.1	2.1	2.3	
B II	20	4.0	4.0	3.9	300 grams meat, 1500 cc. milk added daily	3.9	3.9	3.8	3.8	3.8	3.9	4.2	4.1	4.2	3.9
	21						3.3	2.8	2.8	3.2	3.6	3.2	3.7	3.5	3.5
	22	3.2	3.4	3.6		3.8	3.9	3.5	3.2	3.4	3.3	2.8	3.5	2.7	2.7
	23	2.3	2.3	2.2		2.2	2.4	3.0	3.1	2.8	2.3	2.1	2.2	2.1	1.4
	24					1.9	1.6	1.4	1.3	1.2	1.0				
	25		3.2	3.5		3.9	3.9	3.8	3.6	3.3	3.6	3.2	2.6	2.4	2.7
	Averages	3.2	3.2	3.3		3.1	3.2	3.1	3.0	3.0	3.0	3.1	3.2	3.0	2.8

TABLE 10—*Concluded*

GROUP NUMBER	CASE NUMBER	DAYS BEFORE			THERAPY	DAYS AFTER BEGINNING OF EXPERIMENTAL PERIODS									
		30	20	10		0	10	20	30	40	50	60	70	80	90
Group B. Hookworms not removed—*Concluded*															
B III	26			1.6	Diet not changed; 6 grams ferric ammonium citrate daily	1.9	2.3	3.0	3.7	4.3	4.6	4.8	4.9	4.9	
	27	2.8	2.8	2.7		2.6	2.7	3.7	3.9	3.7	3.4	3.7	5.2	4.7	
	28					3.1	3.5	4.5	4.8	4.6	3.7	4.5	4.2		
	29			2.8		3.2	3.6	3.9	4.0	4.1	4.8	4.8	4.8		
	30					2.4	3.6	3.9	4.0	4.1	4.5	5.0	4.7	4.4	3.7
	31					3.0	3.3	4.3	5.4	5.8	5.9	5.1			
	32					3.6	3.9	4.2	3.9	3.8	3.9	4.3			
	Averages	2.8	2.8	2.4		2.8	3.3	3.9	4.2	4.4	4.4	4.7	4.8	4.7	3.7

carrying capacity of the circulation significantly and rapidly improved, and the symptoms of the anemia consequently alleviated. The average hemoglobin values for the 5 patients of group A-III, who were treated for the parasites and then given daily 6 grams of ferric ammonium citrate, as well as the average hemoglobin values for the 7 patients of group B-III, who received the same amount of iron salt but no treatment for the removal of the hookworms, increased rapidly after the beginning of the administration of the iron ammonium citrate. The average gains of hemoglobin in each group are comparable, and amount to 34 and 38 per cent respectively during the first thirty days of iron therapy. During the first sixty days of iron therapy each group gained 44 per cent of hemoglobin. It is thus clear from these observations that the presence of the parasites had no detectable inhibiting effect upon the production of hemoglobin in response to iron therapy. This result confirms the observations conducted under controlled conditions in the hospital, and demonstrates the practicability of therapy with iron in the field.

VI. DISCUSSION

The observations reported above, concerning the etiology of the anemia of hookworm infestation, at least in Puerto Rico, make it clear that as far as the morphology of the blood picture and of the bone marrow is evidence of similarity, the anemia resembles various types of hypochromic anemia unassociated with hookworm infection. Patients showing no evidence of hookworm infection but with identical blood pictures and physiological responses to hematopoietic substances were encountered in Puerto Rico. Today the hypochromic anemias are considered to be mainly due to defective blood formation, particularly to defective hemoglobin production. Apparently the immediate cause is a lack in the body, mainly of available iron (21). The remarkable effectiveness of large doses of iron ammonium citrate in the relief of the hypochromic anemia of hookworm disease does not differ from that observed in the therapy of other types of hypochromic anemia in which faulty diets, gastrointestinal changes, and blood loss clearly play rôles of varying importance in bringing about an iron deficiency. These possible etiological factors were accordingly evaluated as far as was possible. Strikingly defective diets, some glossitis, a moderate percentage of gastric anacidity, and a good deal of diarrhoea were found. The reduction of available hematopoietic material in the body through blood loss rests upon a firm basis from experiments on animals, and was confirmed by our observations on the hematopoietic effect of injected red blood cells. Since it was impossible to demonstrate any direct inhibitory effect of the parasite upon the function of the bone marrow, it is our belief that the influence of the parasite in the production of anemia depends solely upon its ability to cause chronic blood loss. Blood loss due to *Necator americanus* undoubtedly occurs, although it is probably not so great as that due to *Ancylostoma duodenale.* The difficulty of reconciling the varying degrees of anemia or even apparently perfect health, which may exist in association with a given amount of hookworm infection, is, however, easily surmounted if it is considered that the other factors, which we have examined, play contributory rôles in reducing the available supply of hematopoietic substances in the body. Despite the fact that beans and peas are apparently a good source of iron, the obvious limitation of the diet in animal protein and in vitamins A, B, and D, brings again into prominence the etiological relationship of dietary defects, which were considered important before the discovery of the parasite. The obviously disturbed condition of the gastrointestinal tract of so many of these patients is sufficient to suggest difficulty with the assimilation of hematopoietic substances. Such a disturbance can be shown to exist in other hypochromic anemias, especially when gastric achlorhydria is present. Not all the patients with hypochromic anemia associated with hookworm infection stand entirely apart from patients with sprue, which is also common in Puerto Rico. Our selection was arbitrarily made on the basis of the hypochromic type of blood picture and the finding of ova in the stools, but patients with sprue are encountered with hypochromic blood pictures. The symptoms of sprue strongly suggest an involvement of the intestinal tract in the etiology of the process. The gastrointestinal disturbances and the macrocytic anemia are probably due to a deficiency in the diet of a heat-stable substance associated with many sources of the vitamin B complex (52) (53) (54). Changes in the gastric function and in the intestinal permeability (55) (56) have been demonstrated in sprue; and it is possible that in the dietary background of the sufferers from hypochromic anemia there are defects which have led to changes in the absorptive function of the alimentary canal with respect to iron. In this way certain individuals could become "conditioned" for difficulty in the assimilation of iron, as is clearly the case in other types of hypochromic anemia. The fact that rapid blood production and striking clinical improvement invariably resulted from the addition of iron salts to the basal diet of the patients, indicates that the chief immediate defect leading to the production of the hypochromic anemia is iron. This does not mean that although no significant effect resulted from improvement of the diet alone, dietary defects other than iron may not play a part leading up to the iron deficiency; nor that an increase of the food iron would not be important in preventing the development of the anemia. The presence of other deficiencies is suggested by the fact that a concentrated aqueous extract of liver containing negligible amounts of iron was found to be effective. The possibility of reversing gastrointestinal changes produced by defective diets over long periods of time is obviously more remote than simply overcoming their immediate effects by the administration of large doses of hematopoietic substances. It also is not intended to imply that because the anemia can be totally relieved by the exhibition of iron without removing the parasites, the latter play no part in its production. In varying degrees in different patients the factors of food deficiency, gastrointestinal changes, and blood loss, apparently share the credit for causing this type of hypochromic anemia as in other varieties.

Certain prevailing impressions concerning treatment are contradicted by an analysis of the problem carried out by testing the effect of only *one procedure at a time.* The observation was repeatedly made that the removal of the parasites resulted in little improvement of the blood picture or the clinical condition of the patients sometimes over periods as long as ninety days. In the severely anemic patients who were selected, the supply of hematopoietic substances had already been so greatly reduced that the mere prevention of further blood loss through removing the parasites could perhaps not be expected to bring about remarkable benefit. It is, therefore, possible that our observations do not represent entirely the effectiveness of removing the parasites from less anemic patients. On the other hand, without removal of the parasites, prompt improvement of the blood values and of the clinical condition invariably resulted from the administration of iron salts without other changes in the diet. Thus, it is clear that by direct treatment of the anemia, on which seem to depend the major symptoms of the disease, clinical improvement can be greatly expedited. In the severely anemic patient the administration of anthelmintics may not be unattended with danger, and certainly causes more discomfort than in a patient already restored to health. The importance of measures directed against the parasite is not to be deprecated, but the possibility of a quick return to better health and economic usefulness should aid in securing coöperation in such prophylactic measures as the wearing of shoes, the erection of adequate latrines and the use of better food.

In addition to the cure of the anemia this approach should be useful as a factor in prevention. Since without anemia the presence of the worms is apparently not in itself detrimental to health, it should be possible to employ iron salts as a prophylactic, either alone or preferably in combination with anthelmintics. In patients with little or no anemia the use of improved diets might be more effective than in the already depleted patients who were studied here. It is important to realize, however, that the nutritional state of the patients maintained on a basal diet seemed to be rendered eminently satisfactory by the addition of ferric ammonium citrate alone. For this reason the cost of the treatment necessary to relieve or presumably to prevent the anemia is astonishingly low. A 50 per cent aqueous solution of ferric ammonium citrate (brown scales) was found to keep well in the tropics and could be prepared with very little labor from crystals costing only $2.00 a kilogram. The daily dosage of ferric ammonium citrate for adults should be at least 4 grams, preferably 6 grams for maximum effects. The full amounts should not be given at first, and then only in divided doses after meals. In children smaller doses are desirable. Undoubtedly the prophylactic dose of iron need not be so large or could be administered at greater intervals of time. Observations on this point should be of great practical value. Other preparations of iron are also undoubtedly satisfactory for routine use in correspondingly large dosage. As with other types of hypochromic anemia associated with changes in the gastrointestinal tract, it is probably essential in many instances to continue the administration of iron after the patient has been returned to health, if assimilation of the food iron is not adequate (62). The effect of the liver fractions tested was found to be either negative or inferior to that of iron salts, and their cost far greater. Liver extracts, therefore, appear to offer no advantages over iron in the treatment of this type of hypochromic anemia. It is to be hoped that since the symptomatology of hookworm infection is predominantly that of anemia, the primary importance and simplicity of dealing directly with the anemia will be appreciated by those engaged in the problem.

VII. CONCLUSIONS

1. Based upon a study of 83 patients, the morphological and physiological characteristics of the severe hypochromic anemia associated with hookworm infection in Puerto Rico were found to resemble closely those of other types of hypochromic anemia not associated with hookworm infection.

2. The anemia was considered to be due mainly to insufficient blood production as a result of a deficiency of available iron and other hematopoietic substances in the body.

3. This deficiency is postulated to be produced by *multiple factors*: by defective diets, or indirectly by gastrointestinal changes, or by

blood loss due to the hookworms. Various combinations of these factors are probably involved in different patients.

4. No effect of the presence of the hookworm could be demonstrated other than could be accounted for by its ability to remove blood from the patient.
5. The removal of the hookworms was shown to produce only slight clinical improvement and to have little effect upon hemoglobin production, but considerable effect upon red blood cell production within several weeks.
6. The improvement of the diet, by the daily addition of 1500 cc. of milk and 300 grams of lean beef, was found to have no significant effect upon the anemia.
7. The daily administration of 6 grams of ferric ammonium citrate, *with or without removal of the parasites*, was attended by striking improvement of the blood values and the clinical condition of the patients.
8. The daily administration of a concentrated aqueous extract of liver was shown to have a moderate effect; of a sub-fraction precipitated by 70 per cent alcohol to have a slight effect; and of a sub-fraction soluble in 70 per cent alcohol given either intramuscularly or orally, to have no effect upon blood formation.
9. The practical importance of directing therapy first against the anemia in hookworm disease and secondly against the parasite is pointed out.
10. The medical and economic advantages of using large doses of iron in the prevention and treatment of the anemia of hookworm disease are emphasized.

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THE ETIOLOGY OF LEPROSY[1]

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CONTENTS

Introduction.......... 377
History.......... 378
Early bacteriology.......... 382
  Discovery of *Mycobacterium leprae*.......... 382
  Methods of study.......... 384
  Attempts at cultivation of Hansen's bacillus and isolation of various strains of bacteria from leprous lesions.......... 389
  Chemistry of certain acid-fast bacteria obtained from lepers.......... 416
  Animal experimentation and results.......... 417
Newer knowledge of the bacteriology of leprosy.......... 423
  Critical evaluation of previous knowledge of Hansen's bacillus.......... 423
  Methods of study.......... 430
  Cultivation of *Mycobacterium leprae* on solid media.......... 432
  Cultivation of *Mycobacterium leprae* in tissue culture.......... 453
  Animal experiments and results.......... 455
  Chemistry of "*Mycobacterium leprae*".......... 473
Summary.......... 478

INTRODUCTION

There is perhaps no problem in human medicine which has been the subject of more acrimonious debate than leprosy. This has been particularly true in relation to the question of its etiology, though the clinic has also been the stage for contention down through the decades. The problems surrounding the study of this most dreaded disease have been the subject of international conferences and, largely because of the world-wide prevalence of the disease and the different manifestations of the infection in various parts of the world, it is not surprising that well defined differences of opinion should exist. These have existed at various intervals and at times have become almost polemic in character. Unfortunately such controversies frequently sublimate points of view on an emotional basis, scientific and intellectual considerations becoming secondary, and the progress of discovery is impeded because the mind is closed. However, a careful review of the literature on leprosy during the past sixty years of investigation reveals that, in spite of the highly controversial nature of the discussions regarding this disease, some definite progress has been made. It will be the purpose of this review to trace the development of our knowledge of this disease and to attempt to present a picture of the present status of its etiology.

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HISTORY

Leprosy is one of the oldest diseases known to man. In this ancient heritage it ranks with at least two other diseases, namely syphilis and trachoma. Garrison (1) states that the outstanding achievement of the Assyro-Babylonians in public hygiene was their perception of the transmissibility of leprosy, which resulted in the expulsion of lepers from the community. Sudhoff (2) has presented records of a formula proclaiming outlawry as stating "transmitted to us upon the stone landmarks of Babylon over 3500 years ago." In the old and new testaments of the Bible one finds no less than one hundred and seventy-three verses devoted to the subject of leprosy. These references date over a period of some fifteen hundred years of Biblical history from the year 1491 B.C. to the year 33 A.D. The first Biblical reference to the disease occurs in the fourth chapter of Exodus, verses six, seven and eight where God sent Moses to deliver Israel, and the Almighty gave to Moses the three signs that he, Moses, was indeed following instructions from God, one of these signs being the power of God to give leprosy to Moses and as rapidly remove the disease from him. So Moses became the first recorded leper in the chronicles of the Bible, though only, we are to believe, for a brief moment. In the fifty-nine verses of the thirteenth chapter of Leviticus there appears a detailed description of what was thought of at that time as leprosy. In the following chapter some fifty-seven verses are devoted to the "law of the leper" given by the Lord to Moses. In this chapter the methods of cleansing the leper of his disease are described as well as the destruction of leper houses and the disposal of unclean materials. In the many other Biblical references to this disease astonishing miracles are frequently described in the form of cures or cleansing of the disease (see Matthew 8:2, 3; 10:8; Luke 4:27; 5:12, 13; etc.) or even the almost instantaneous appearance of the disease (see Numbers 12:10 where Aaron looked upon Miriam as the cloud departed from off the tabernacle and behold she was leprous). In other references direct evidence is found leading us to believe that in those days leprosy was regarded as a curse which hereditarily affected the issues of man (see Numbers 5:2; Samuel 3:29; II Kings 5:27 where it is said that "the leprosy therefore of Naaman shall cleave unto thee, and unto thy seed for ever.")

Down through the ages this story of a plague known as leprosy, and given so much attention in the Biblical history of mankind, has established in the minds of the various peoples of the earth the idea that leprosy is a disease to abhor; that it is a curse to man and his children; that its presence provokes profound social implications necessitating that its victim should be segregated from his fellow man; that it is associated with filth and poverty and all that is unclean and detestable; in short that the leper is a *thing to be avoided* and a thing to be cast aside, segregated by force if necessary or utterly destroyed so as to remove *it* from sight and the thought of the human mind. History depicts nothing more tragic than the leper.

One of the most frequent questions raised by the lay public in our present day so-called enlightened civilization is whether this plague described as leprosy in Biblical history could in reality have been the actual disease of leprosy as it has been recognized in more recent periods of history. Many are the authorities who are unwilling to accept the Biblical description of the plague as a true picture of clinical leprosy and, as Garrison states, "Modern dermatologists contend . . . . that Biblical leprosy (zaraath), of which Naaman was healed by dipping himself 'seven times in the Jordan,' and which was transferred (in the folk-lore sense) to Gehazi, so that 'he went from his presence a leper as white as snow' was, in reality, psoriasis." It is doubtless true that the clinical picture of the plague described in Leviticus corresponds only in a very vague way with clinical leprosy, as we know it, and the diagnosis of this plague as psoriasis by dermatologists seems a most satisfactory and accurate one.

It must be recalled, however, that historians are not entirely to blame for having recorded this plague as leprosy and, indeed, there may have been cases of true leprosy before the second book of Moses of the Old Testament, though no mention of such a disease is made in the book of Genesis. As students of the history of medicine we should probably be content to be able to trace back fairly definitely a disease, such as leprosy, which is the subject of our interest here, some thirty-five hundred years. Let us turn then to more modern records where the terrain is quite certain.

Certainly our knowledge of the existence of this disease many centuries back is indisputable. We have available written records of such technical observations as the symptom of anaesthesia as far back as the fourteenth century and even in this time it was suggested that leprosy is contagious and manuscripts contained records of the civil status of supposed lepers. Walsh (3) indicated, in his translation of "The Popes and Science," that Emperor Frederick II in 1224 A.D. issued an edict regarding the practice of medicine and one of the duties of the physicians of that time was to determine the diagnosis of leprosy because of the civil status of such patients. The existence of leprosy then assisted the physician in establishing an important place for himself in society and with the State, just as the existence of this disease assisted greatly in the development of hospitals, due to its wide prevalence. We are told by historians that in the fifteenth century people having such diseases as leprosy, trachoma, anthrax, bubonic plague, and a few others were not permitted to enter cities or were isolated or driven from cities, and such persons were not allowed to handle food and drink for sale or transport by sale. By this time books and other manuscripts had appeared with descriptions and illustrations of such diseases as leprosy, some old and some modern for the time. By the middle of the sixteenth century many of the epidemic diseases were abating in parts of Europe. Among these, as we are told, was leprosy, though the disease continued epidemic in Germany, Scotland and the Low Countries and persisted throughout the seventeenth century in these places and into the following century in Norway and Sweden. Leprosy was becoming so rare during these periods that the old lazar-houses were abolished in many parts of Europe. Garrison recalls to mind that "Relics of the disease in art are preserved in Ruben's painting of St. Martin (Windsor Castle) and Murillo's St. Elizabeth in the Prado (Madrid)." Recently the writer came upon a painting by Ferdinand Bol in Amsterdam of "The Four Governors of the Leper Colony" which is considered the masterpiece of this pupil of Rembrandt and was painted in 1649. Van Dyck has also depicted leprosy in his "St. Martin Dividing his Cloak."

The beginning of modern dermatology is said to date from the unfinished work of Willan "On Cutaneous Diseases" (1796–1808) which was completed by his pupil Bateman and psoriasis (Biblical "leprosy" of Gehazi and Naaman) was more clearly defined and differentiated in this notable work on skin diseases. It was at about this period that we find the beginning of tropical medicine, the general field in which leprosy is usually placed today. Following the development of the East India Company, chartered by Queen Elizabeth in 1600, there was a growing interest in Far Eastern medicine. During the eighteenth and nineteenth centuries there were many treatises published on Indian climate and diseases. The early treatise on tropical medicine by James Lind (1716–1794) in 1768 was the real beginning of tropical medicine as a subject. From this time on we find more accurate records and descriptions of such diseases as leprosy, though over one hundred years was yet to elapse before Hansen observed in leprotic lesions the bacillus which bears his name. Later on in this so-called modern period of medical history we come to the genesis of the science of bacteriology and it is within this science that we find the etiology of leprosy, though various theories other than bacterial were advanced and held for many years, as we shall see, regarding the etiology of this disease. We need only mention at this point such theories as the fish theory and the scurvy theory, both of which had their proponents in their day.

In summarizing the history of leprosy we may state that it is generally conceded that this disease was known by the ancient Chinese, Indians and Egyptians. That Biblical "leprosy" was most probably psoriasis and not real leprosy is also generally recognized. Leprosy was probably introduced into Greece three or four hundred years before Christ and by the seventh century was quite prevalent in Southern Europe. Manson-Bahr (4) states that leprosy was introduced into England about the year 950 and he states that the last British leper died in Shetland in 1798. At present the disease is quite widely distributed in subtropical and tropical countries though it also occurs to some extent in colder climates. In 1932 we (5) stated that it was estimated that there are from one to four million lepers in the world today, but that the exact number is unknown. It is possible that if the total number of cases of this disease were known it would be twice the latter figure. Widespread occurrence of the disease is found in India, China and the Philippines and certain parts of Africa today. The disease still lingers in several other localities such as Puerto Rico, the Virgin Islands, parts of South and Central America, several of the European countries and in the United States itself. That leprosy still remains an important public health problem there can be no doubt and its economic loss is a cogent problem which every country having lepers within its borders must take into account. The continued study of its etiological agent and the discovery of new knowledge leading to better methods of treatment and control are therefore most important.

EARLY BACTERIOLOGY

Mycobacterium leprae was first described by G. Armauer Hansen (6) in 1874. Hansen first noted rod-like organisms, which he thought were bacilli, in the cells of freshly excised lepromata. At this time staining methods were not available to Hansen and it was not until 1880 that he (7) (8) was able to apply these methods to the organism. In these latter papers Hansen discusses the claims of Neisser and of Edlund, both of whom had visited his laboratory and later published their views that the organism of leprosy is a micrococcus (Edlund) and a bacillus (Neisser). Hansen stated his case for priority of discovery of the bacillus of leprosy pointing out that Edlund's observation of micrococci in the blood of lepers was unreliable. This was the age of bacteriology and the beginning of an entirely new line of investigation in the study of disease. Hansen, at this time, reported a careful study of a case of leprosy from which he obtained scrapings from tuberculous lesions and in this material he demonstrated bacilli which were stained with methyl violet, a method which was suggested to him by Koch. By repeated experiments with this method, and in particular by lengthening the time of staining and employing higher concentrations of the dye, Hansen eventually obtained well stained organisms. A plate of diagrammatic drawings by Hansen illustrates the morphology of the organism and shows variations from typical rod-like structures to chains of coccoid forms, as well as a peculiar grouping of the organisms now spoken of as globi. During the same year Hillairet and Gaucher (9) published a short review on the etiology of leprosy and claimed to have demonstrated organisms in the blood of lepers. By attempting cultivation in blood these authors described a filamentous growth resembling mycelia which now can most certainly be regarded as contaminants. A word of caution was introduced into the records at this same time by Besnier (10) who published a paper on the contagiousness of leprosy. In commenting on Hansen's observations this author stated that the time was premature to accept them as proven facts. A further report by Harris (11) in 1880 describes certain microscopic preparations of leprosy tissue, but no mention is made of finding bacteria in these tissues. A similar study was presented the same year by Caley, Liveing, Duckworth and Powell (12) in which no mention was made of bacteria in any of the tissues studied by them.

These few papers stimulated great interest in the study of the etiology of leprosy. For the next fifty years this organism observed by Hansen was to be a subject for debate, particularly with regard to the question of its artificial cultivation, for the Hansen bacillus proved to be an organism which was to defy the ordinary methods employed to cultivate bacteria and, indeed, it has seemed that in this organism we have had the ideal obligative parasite. As we shall see later, however, hopes of actual cultivation *in vitro* of *Mycobacterium leprae* are not entirely given up and there is evidence accumulating that it has been finally successfully cultivated upon artificial medium.

The work of Hansen was soon confirmed by various investigators. In 1881 Cornil (13) published a general discussion of the bacteriology of leprosy and described the organism of Hansen. Again, in another paper, Cornil and Suchard (14) confirmed the finding of Hansen's bacillus in the tissues of lepers and suggested that its distribution between the fibers of the tissue constituted a barrier against diffusion of the microbe to the outside and they postulated that this accounted for the difficulty of contagion. Their colored illustrations leave little doubt that they were most probably dealing with the true organism of leprosy observed originally by Hansen. In an additional report Cornil (15) described leprous tissues from a cutaneous tubercle, lymphatic glands, the cornea, larynx, liver, testicle and cubital nerves and stated that the leprosy organism differs in size in the various locations, it being five or six times the size in testicular tissue as compared with its size in the skin. From these few early papers dealing with the presence of a bacillary form in leprous tissue and the distribution of this organism in the tissues we can readily see that the methods of study were both subjective and objective at this period. As the science of bacteriology was extended other methods of study were applied to the problem and the trend of this development can perhaps be best illustrated beyond this point by mention of further landmarks in the study of this disease.

Methods of study

Several of the early papers dealing with the etiology of leprosy, such as those by Bordier (16), Shuliovski (17), Herando (18), etc., it will be necessary to pass over with little comment except to state that they indicate a growing interest for the time in this subject and many papers such as these were presented in various countries by way of bringing to the attention of physicians newer knowledge of the malady. While Hansen first attempted cultivation of his organism without success we find that in 1881 Gaucher (19) obtained some culture media and the use of an incubator from Bouchard and reported that in the blood of lepers he observed freely moving organisms of various forms which he was able to cultivate in Liebig's bouillon after three days incubation at 36° to 40°C. In culture his organisms were found to be micrococci and were observed both singly and in chains. These organisms were without doubt contaminants. In 1882 Thin (20) published a review of the recent literature dealing with leprosy and offered the observation that Hansen's bacillus had up to that time been found in several parts of the world or in tissues from different localities. He described a study of a case of leprosy in a Hindoostani and stated that the bacilli are to be found always in cells as small as white blood cells and in lymphocytes, suggesting that the disease might be spread through the lymph cells. The beaded appearance of the organisms is mentioned in this report and it is the first record we have been able to find where mention is made that the leprosy bacillus retains fuchsin following the action of dilute nitric acid, a method still employed in the study of this organism. A year later Thin and Hillis (21) published a short note describing the leprosy bacillus in the skin of a negro. Hansen (22) had in 1882 published a report on attempted cultivation of *Mycobacterium leprae* in gelatin and solidified blood serum, as suggested to him by Koch, and obtained some long filaments composed of several bacilli. After five days, however, he states that the bacilli were transformed to "grains" (no doubt meaning granular forms). He inoculated a monkey with leprous tubercle material, but the material was completely absorbed. Later he injected the ear of a monkey with his supposed culture of *Mycobacterium leprae* but after four months there remained no evidence of the injection. In the same paper Hansen reported finding no organisms in two specimens of the anaesthetic form of the disease.

About this time three papers appeared in the United States by Belfield (23), Bermann (24) and Schmidt (25). The former, besides reviewing the literature up to that time, mentioned the introduction of staining methods by Weigert and Koch, the use of the Abbé condenser and the employment of culture methods. He recalled the inability of Neisser (26) to demonstrate the organism in the blood and the negative findings of Schmidt who did not employ stains. He also commented on Neisser's experiments with dogs and rabbits in which nodular lesions were produced and introduced a pertinent aphorism that we should heed even today, that "one nodule does not make a leprosy." In two cases of leprosy Belfield was able to demonstrate Hansen's bacillus in the tissues. Bermann also reported success in finding the organisms in tissues. By this time there was little question remaining regarding the validity of Hansen's organism. In 1882 another paper came from Hansen (27) reporting negative results following the injection of rabbits and cats with leprosy material and including a reference to negative results obtained by Köbner (28) in both monkeys and fish. At this time Hansen remarked that leprosy is "cette maladie énigmatique." How true this was (and still is) is brought out in a few papers which followed this paper of Hansen by such authors as Planellas (29), Babes (30), Barduzzi (31), Moretti (32) and Baumgarten (33), all of whom reviewed the general subject in the following year and arrived at no definite conclusions. In 1884 we come upon another paper by Thin (34) and one by that romantic figure in tropical medicine, Patrick Manson (35). Thin studied the larynx of a case of leprosy and employed fuchsin and methylene blue in staining microscopic sections of this organ. He presented descriptions and drawings of cells and bacilli and suggested that the white blood cells containing the bacilli are carried by the circulation to different parts of the body, finally leaving the vessels to produce masses of lepra-cells leading to the destruction of fibrous tissue and a low grade of inflammation, followed again by an exudation of cells and the formation of new fibrous tissue. Already, we see, the mechanism of infection was becoming a subject of interest. Manson's paper was in the form of a letter to the Editor of the *Lancet*, sent from Hong Kong, in which Manson mentioned the earlier discovery of the leprosy bacillus in comparison with *B. tuberculosis*, but pointed out that the diagnosis of leprosy was still difficult and somewhat impractical. He suggested the method of squeezing the leprosy nodule until the blood is driven from it and then pricking the nodule with a needle to obtain pure exudate on cover-slips, which could then be dried and stained. He stated that he had found this method easy, expeditious and a reliable way to diagnose leprosy. While simple staining methods had been introduced some years before, these, as well as new methods, now became the subject of extended study by several histopathologists, notably Unna, who was to contribute many reports over a period of years dealing with staining and the histopathology of leprosy. In 1885 Unna (36) published an interesting summary of staining methods and a study of the histopathology of the disease with plates illustrating some different types of the infection. During the same year Stevens (37) conducted a fairly comprehensive review of the literature, including data on staining methods, presented a study of the skin of a typical leper, and concluded that leprosy is entirely dependent upon the presence and development of a specific organic virus. The observations covered in several papers which followed by Jakowski (38), Guttmann (39), Unna (40), Touton (41), (42), Neisser (43), Hansen (44), Arning (45), Unna (46), (47), Schottelius (48), Lutz (49) and Touton (50) were devoted to the distribution and location of the bacilli in tissues and to the arrangement of the bacilli. Unna described the peculiar clumping of the organisms, Hansen applied the Gram stain to the bacilli while Arning searched for the organisms in the nerve type of the disease. Early in 1886 an editorial in the New York Medical Record (51) described the inoculation of a native named Keanu, a condemned criminal in the Hawaiian Islands, with leprous material. Arning injected this material in the arm of the prisoner and fourteen months later the bacilli were found at the site of inoculation, but there were no constitutional symptoms of the disease. This same author found that in putrid leprous tissues or in the body of a leper who had been dead for three months, the bacilli were to be found in great numbers. A study on morphology of the organism by Lutz (52) is of some interest at this time since he described and presented sketches of single and double club forms, coccoid forms and chains of these peculiar rather large round forms with rod-like tails and thread-like masses, all of which he believed represented various forms of the leprosy organism and illustrative of the pleomorphism of this microbe. Rake (53) presented his opinion that *Mycobacterium leprae* is contained within the cells as opposed to that of Unna who stated that the organisms were only clumped in the connective-tissue spaces, a view also held by Griffith (54) who said the organisms were held together by a mucoid material, though admitting that he found organisms in the cells in one case sent from Vienna and examined by him. A paper by Destrée (55) is of interest since he presented an historical account of the disease, with a summary of knowledge dealing with the anaesthetic and tuberculous forms, in which he made a plea for a more humane treatment of the leper and for better hygienic care. In a series of papers Ferré (56) (57) (58) concludes that the finding of *Mycobacterium leprae* in the blood stream of lepers is rare but that it may be found in a certain number of cases. The question of mode of spread of the infection was definitely in the minds of investigators of that period and such studies as that of Chassiotis (59) in 1887 who described *Mycobacterium leprae* in the tissues of the spinal cord, and of Binder (60), only tended to sharpen this interest.

In 1888 an interesting study on attempts to cultivate *Mycobacterium leprae* was reported by Rake (61). Rake employed various types of nutrient media including blood serum, serum from the chest, abdomen, tunica vaginalis, mixtures of serum with gelatin or agar and ascitic fluid. For inoculum he used cutaneous nodules, lung nodules, pieces of viscera, nerve tissue, fluid from blisters, blood and femoral glands of lepers. In addition he employed tissues where degradation products were plentiful, (and which were to be claimed over twenty years later to be so essential for the cultivation of the leprosy bacillus) and only from these did Rake obtain any cultures. In fragments from such putrescent tissues he obtained whitish growth like drops of oil paint, smooth oily, canary-yellow growth, salmon colored growth, faint white growth and states that common molds were often present. In these various growths he found cocci, streptococci, large rods and small rods. He concludes that his various experiments failed to lead to the cultivation of the leprosy bacillus. During the same year Bordoni-Uffreduzzi (62) reported the cultivation of a diphtheroid from leprosy post mortem tissues in peptone-glycerin-blood serum, but the inoculations of this organism into guinea pigs, rabbits and mice were all negative.

At this time there were still those who doubted that leprosy is contagious and failure to cultivate the organism and to induce the disease in lower animals did not assist in convincing such individuals otherwise. In 1889 Stallard (63) concluded that leprosy is only feebly contagious, less so than tuberculosis, but that the spread of leprosy in the Sandwich Islands stood as absolute proof that the disease is contagious. He thought that its spread in these islands was probably due to new conditions of life brought about by association with white people. This view regarding contagion of the disease was concurred in by Pereira (64). By this time nearly fifteen years had elapsed since the discovery of *Mycobacterium leprae* by Hansen. A few years hence the first International Leprosy Conference was to be held in Berlin (1897) but during the intervening years much was yet to be written about this disease. Neisser (65) (66) continued the study by introducing new methods for staining the leprosy bacillus and suggested the use of rosanilin and pararosanilin. He raised the question of the primary or secondary importance of the coccoid forms of the organism in the mechanism of infection. Several papers still dealt with the distribution of the organism in the various tissues, notably those of Giantruco (67), Wynne (68), and Rake (69). Rake, in his reply to Wynne, stated that the bacilli are to be found both in and out of cells and, on the basis of ninety-three autopsies, that the organisms are not found in the blood, in ulcers, necrosed bone or in anaesthetic patches but may be found in the medium nerve. He further reported that the organisms were most common in tuberculous and mixed forms of the disease. Quinquand (70) stated that the earliest manifestation of the disease is a macule. In Russia a general review of the subject was contributed by Bhykoba (71). Unna (72), Favrat and Christmann (73), Delépine (74), Risso (75), Thin (76), Strazza (77), Petrini (78), Fisichella (79), Campana (80), Bodin (81), Unna (82), Gravagna (83), Stephan (84), Wolff (85), Petrini (86) and others continued studies over the next few years on staining methods for demonstrating *Mycobacterium leprae*, distribution of the organism in the tissues and complicating infections. Delépine's study was interesting in that he demonstrated the leprosy bacillus in the sputum, bronchial tubercles, mucous membrane of the trachea and larynx, striped muscle, spleen, phalangeal joint, bone (phalynx), around the vessels, Haversian spaces, intestinal ulcers, liver and in the skin from various parts of the body. Thin described tuberculosis complicating leprosy which was one of the earliest recognitions of this complication. Strazza found *Mycobacterium leprae* in the vocal cords. Campana described a secondary infection in a case of leprosy with a streptococcus. Unna pointed to the fatty substance in *Mycobacterium leprae* and *B. tuberculosis* as differentiating them from other forms of bacteria. Stephan reported finding *Mycobacterium leprae* in the blood stream of a case of leprosy of the anaesthetic type. This was confirmed by Wolff.

Meanwhile other investigators were busy with the cultivation problem and the theories concerning the etiology of the disease. It is quite apparent that during these several years following the discovery of Hansen's bacillus investigators were concerned chiefly with demonstrating the presence of the organism in the tissues of lepers to the satisfaction of everybody. Methods of study were few but, as we shall see, opinion regarding the etiology of leprosy was gradually crystallizing. This period definitely belonged to the pathologist and a summary of the information at hand early in the nineties of the past century indicates that much credit is due to him.

Attempts at cultivation of Hansen's bacillus

In the preceding section mention has been made of some of the early work in attempts to cultivate *Mycobacterium leprae*, including the early experiments of Hansen himself. We have seen that such other studies as those conducted by Gaucher, Bordoni-Uffreduzzi, Babes, Boinet, Cornil, Helcher and Ortmann, and Giantruco did not lead to the successful cultivation of the true Hansen bacillus. In 1889 Campana (87) published a short note on the cultivation of *Mycobacterium leprae* which he offered with some reluctance as a tentative report. Kanthack and Barclay (88) (89) in 1891 reviewed the work on cultivation up to that time, reported their own experiments with culture media employed in hanging drop and in Roux tubes (glycerin-agar), and concluded that they had met with little success. During the same year Campana (90) published another paper no more convincing on cultivation than the first. This was followed by a paper by Ducrey (91) in 1892 who claimed positive cultivation of *Mycobacterium leprae* in glycerin broth and also in stab cultures of solid media in hermetically sealed tubes. Ducrey concluded that *Mycobacterium leprae* is an anaerobe. Campana (92) (93) (94) then in a series of papers on cultivation described his further efforts using neutral medium of broth and peptone to which five per cent agar and three per cent of grape sugar were added. He stated that he obtained in from seven to nine days in the depths of the medium, growth of organisms which were slightly acid-fast and contained acid-fast granules. Byron (95) also reported a pure culture of the organism in 1892 which remained unconfirmed. A similar claim came from the laboratory of Rocca (96).

While these various reports were coming into the literature the various theories regarding the etiology of leprosy were still being widely discussed and stimulated further work on attempts at cultivation of Hansen's bacillus. The so-called fish theory has already been mentioned. The chief proponent of this theory was Jonathan Hutchinson. Others held that the disease was hereditary while a growing number of investigators believed in the contagious theory. As early as 1889 Abraham (97) reviewed these theories and brought together some pertinent information bearing upon them. He stated that Hutchinson admitted that leprosy occurs in immigrants as well as in leper families, but that he still adhered to the fish theory, particularly that the consumption of decomposed or salted fish was responsible. Abraham mentioned Dr. Blanc's observations of 42 lepers in the

United States (of whom seven were German, one Austrian, one English, one Irish, one Italian) with eighteen remaining children German and Irish born. He also mentioned Hansen's trip to America where he found in Wisconsin, Minnesota and the Dakotas, records of 160 lepers with thirteen descendants remaining none of whom had leprosy. Abraham also brings out the fact that many people who eat fish do not develop leprosy and recalls the observation of Dr. Thalozan in Persia, who stated that there was little leprosy in the lowlands of Persia where fish is consumed but much of the disease in the mountains of Kurdistan where the people do not eat fish. Summing up the opinions of the time Abraham stated that Hansen, Sand (Trondhjem), Kaurin (Molde), Philipp (Jamaica), Saunders (Jamaica), Blanc (New Orleans) and Fox (New York) all favored the contagion theory while Hutchinson, Danielson, Nickoll and Fitch (Honolulu) favored the non-contagion point of view. He further states in this treatise that Rake (Trinidad) favors neither theory—that his animal inoculations were negative—that he believes that Arning had not conclusively demonstrated human transmission. Bangilli and Profeta (Sicily) had also reported negative human transmission experiments. Abraham recalls to mind also that in 1867 a questionnaire had been sent to 250 workers in leprosy and the vast majority, at that time, did not believe in the contagion theory though thirty-two did favor it. The Royal College of Physicians at that time paid little attention to the minority but twenty years later, in 1887, the Leprosy Committee Report of the Royal College admitted the *possibility* of contagion.

A second paper of great interest in this period, and one which doubtless stimulated investigators to further effort in attempts to cultivate *Mycobacterium leprae*, was a review by Unna (98) in which he stated that the core of the leprosy bacillus consists of a row of granules comparable to free cocci, that these are surrounded by a capsule and that a row of three, four or eight of these granules in a capsule resemble a rosary (Leitz called these "cocothrix"). Both Unna and Leitz suggested that *Mycobacterium leprae* holds a position between cocci and bacilli. Unna also described, for the first time, an inner capsule surrounded by the older capsule running tangentially over the cocci to give the picture of a rod. Then, he states, the bacillus is covered by a glassy, mucous substance which holds many together as a clump or "zooglea." Therefore he states there are no spores and the nucleus of the bacillus consists of elements equivalent to these cocci, and he goes on to say that the unstained spaces are not signs of degeneration but a step in the normal development of the bacillus. Present-day advocates of theories regarding states in the cyclogeny of bacterial cells would find comfort no doubt in these early views of Unna. Unna also commented on the nature of the so-called lepra cell and presented the view that it does not consist of animal cell protoplasm but of vegetable mucous of *Mycobacterium leprae* origin. He thought that the gloea permeated the entire tissue in the direction of the lymph spaces between the fibers and cells of connective tissue, between cells of the prickle layer and the hair follicle, and filled up the lymph spaces with sausage-shaped masses, leading to proliferation of connective tissue cells quite apart from any process of inflammation. He concluded that only in acute eruptions is inflammation present and that one half to three fourths of skin lepromata and nerve lepromata in substance consists of organisms.

To return to the question of cultivation let us recall to mind that up to the time of the International Leprosy Conference of Berlin in 1897 there were few claims in the record for the actual cultivation of *Mycobacterium leprae*. Only claims for cultivable diphtheroids and possible anaerobes had been made. No acid-fast chromogens or non-acid-fast chromogens, with the exception of those described by Rake obtained from putrescent tissues, had been described. Rake himself did not seriously consider these organisms to be related to the disease. In 1897, however, Lévy (99) reported the growth of a diphtheroid from a leprous nodule, upon glycerin agar having a film of human blood serum. Growth took place in fifteen days but inoculations into rabbits, guinea pigs and mice were negative. In the next two years similar diphtheroids were isolated and cultivated from leprosy material by Spronck (100) who obtained growth on glycerin-potato, blood serum and agar, and demonstrated specific agglutination with leper serum in rather high dilutions (1:1000); by Czaplewski (101) who obtained similar organisms from the nasal secretions and from an ulcerated nodule, and produced cultures on sheep's blood serum with glycerin, but reported only negative results following inoculations into rabbits, guinea pigs and mice; and by Teich (102) who cultivated diphtheroids from nasal secretions from lepers.

At the International Leprosy Conference in Berlin great interest was apparently manifested in the subject of leprosy. Among the most interesting papers contained in the volume devoted to the transactions of this Conference is that of Neisser (103) who contributed a review dealing with some of the problems in leprosy; of Herman (104), who discussed the leprosy bacillus at different periods of its growth in the human body; of Aristidi Bey (105); and of Ashmead (106) who offered an interesting theory concerning the development of leprosy as a disease. Ahsmead stated that leprosy supposedly originated in Central Africa. He postulated a bacillus which originally was no offender against society and which suddenly (or gradually) underwent "variation" as man himself has done. In other words the organism became virulent and began to ravage mankind. He stated that after such a disease as leprosy has ravaged a race for some time it loses its virulence, and he argued an analogy in the case of syphilis which, after 400 years in Europe, is found in a different degree than in Japan after 1300 years of existence there. He states that in Japan in the richer classes leprosy is rare, in the middle class it is more frequent and in the poor class it is common. He suggested that varying degrees of inbreeding favor immunity to the disease.

For the next three years, or until 1900, nothing of importance was reported in regard to cultivation work on *Mycobacterium leprae*. During this time, however, interest in the general subject of leprosy did not wane as is illustrated by papers by Babes (107); Weber (108), who reported finding *Mycobacterium leprae* in human sperm; Unna (109) on staining; Schäffer (110); Riatti (111), who described bacilli in the spinal ganglia; Campana (112); Scanga (113), who transplanted leprosy material into the brains of pigeons and produced large accumulations of organisms in that locality; Calabrese (114), who observed *Mycobacterium leprae* in the urine of a leper having nephritis; Bergengrün (115); Babes (116); Winfield (117) who reported a case of leprosy in Key West, the uncle and father having died of the disease; Tashiro (118); Shibayama (119); Jeanselme (120) (121) on localization of organisms in the tissues; Horder (122) on the finding of *Mycobacterium leprae* in the blood; Bordoni-Uffreduzzi (123); Brannikow (124) (125) (126) (127); Pernet (128), who described *Mycobacterium leprae* in cells but stated that the majority are outside the cells; Uhlenhuth (129); Woit (130) who described *Mycobacterium leprae* in the maculo-anaesthetic type of the disease; and Fraenkel (131) who published an atlas showing the various lesions of leprosy. In 1898 Babes (132) published a treatise of ten chapters on leprosy dealing chiefly with the pathologic anatomy of the disease and a study of the presence of *Mycobacterium leprae* in various tissues of the body. Two years later this author (133) suggested the presence of a toxin elaborated by the leprosy bacillus and stated that he believed some of the general and local symptoms of the disease, particularly referable to the central nervous system, were due to such a toxin. During the same year Scholtz and Klingmüller (134) reported their work on cultivation of *Mycobacterium leprae* and concluded that no report on cultivation to date could be accepted as free from doubt and that the organisms previously described probably have nothing to do with leprosy. These authors employed as media agar, glycerin agar, ascitic agar, maltose and grape sugar agar, blood agar, potatoes, gelatin, bouillon, and ascitic broth; they isolated no germ which they felt they could call *Mycobacterium leprae*. Also they found no substance analogous to tuberculin which they could extract from *Mycobacterium leprae*.

In 1900 and 1901 we come to the cultivation work of Kedrowski (135) who also described an organism which we must classify with some of those previously reported, as diphtheroids. Kedrowski isolated this organism from leprosy tissue in two cases and from an abscess of a leper. The organism was grown on placenta extract agar but later subcultured on plain agar and upon glycerin agar. Young cultures were acid-fast but as they became older they lost their acid-fastness, except for their granules. Kedrowski inoculated rabbits and claimed to have recovered the organisms from these animals several months later. This work left the problem of cultivation of the causative agent in leprosy about as it had stood in the past but although unsuccessful it served to keep interest alert and hope alive that eventually the problem would be solved satisfactorily.

For the next few years, until 1905, little was reported along the lines of cultivating the organism of leprosy but contributions were made to the literature in other fields of the subject. In passing we may mention reports by Gerber (136); Barannikow (137) (138); Azzarello (139), on examination of the blood for *Mycobacterium leprae* and the inoculation of animals with leprous material which experiments proved to be negative; Leredde and Pautrier (140), who reported the advantages of the examination of leper's nasal mucous discharge after ingestion of potassium iodide; Hutchinson (141), who reported a conversation with Hansen in which the latter admitted that *Mycobacterium leprae* could not be distinguished from *B. tuberculosis*, that the former occur in great masses while the latter do not, that the latter has a potent toxin while the former does not, that *Mycobacterium leprae* cannot be cultivated, that the disease in Norway began to decline when intermarriage was prohibited; Shoemaker (142), who reported a case of leprosy with bacilli in the circulating blood (in leucocytes and free in the plasma as well); Boston (143) (144) (145), who also reported leprosy bacilli in the leucocytes of the blood; McFarland (146), who concluded that the bacillus of leprosy is a pure parasite; Daland (147) (148), who reviewed the history and status of the disease in Japan and Norway and discussed theories regarding method of spread, such as through wounds in the skin, through bare feet, etc.; Ivanoff (149); Hynbert (150) (151) (152); Van Houtum (153) (154); Hallopeau (155), who described *Mycobacterium leprae* in exostoses of the bones and stated that lepers are prone to have these growths; Hynbert (156); Taormina (157); Zenoni (158), who published a general review of the histopathology of nodular leprosy; Emile-Weil (159), on staining reactions of *Mycobacterium leprae*; Hallopeau and Grandchamp (160), who described two cases of erythematous leprosy as lesions distinct from bacillary lesions; Goodhue (161) (162), who published a history of leprosy in Hawaii from its origin in those islands in 1863 up to the time when *Mycobacterium leprae* was found in mosquitoes and in bed bugs—also calling attention to the fact that the disease was known in Hawaii as "mai poke" and as "Chinese disease"; and Stalberg (163) on the pathologic anatomy of the disease.

On returning again to the question of cultivation we find a report by Karlinski (164) in 1903 briefly stating that he had cultivated *Mycobacterium leprae* from three serums from three different patients. Later, in 1912, this author was to report the cultivation of an acid-fast non-pigmented organism from leprosy tissue which he was able to keep alive for several months. During the same year (1903) Kedrowski (165) published another paper on cultivation of *Mycobac-*

terium leprae and reaffirmed his belief that he had succeeded in cultivating the germ of leprosy. Guarch (166) at this time reviewed most of the cultivation work to date but added nothing new to the picture. In 1904 Rost (167) in Rangoon reported that he had succeeded in growing *Mycobacterium leprae* by withdrawing the salts and chlorine from his beef medium and found that *Mycobacterium leprae* grew as a white, slightly yellow, stringy material, particularly yellow on solid media when the salts are removed. He states that in dialysed beef broth and fresh broth the organism grew well. He classified the acid-fast bacilli as the "achloretic group" comprising *Mycobacterium leprae*, *B. tuberculosis* and *B. lustgarten* and stated that in his medium *Mycobacterium leprae* tended to stick to the bottom more than the other two. With his cultures he prepared his so-called "leprolin" with which he treated lepers. He stated that sensation returned to the anaesthetic spots or patches and some nodules exhibited signs of breaking down. His leprolin was prepared by growing his organism in distilled beef extract for six weeks at 37°C., then sterilizing and passing through a Pasteur filter, after which glycerin was added to the filtrate. No animal inoculations were reported by Rost with his supposed *Mycobacterium leprae* culture.

The following year Weil (168) (169) described the cultivation of *Mycobacterium leprae* from tubercular leprosy with colony formation on egg medium. The same year Klitin (170) (171) cultivated organisms from four cases of leprosy, employing excised nodules, and described forms similar to *B. tuberculosis*, though shorter and more granular. These should be added to the list of diphtheroids described heretofore. This author states that his cultures produced lesions in rabbits and guinea pigs and that it was possible to recover the organisms from the lesions produced in these animals. About the same time Turner (172) reported that he had attempted to repeat Rost's work on cultivation but without success. Rost, who had been receiving a great amount of criticism concerning his work, was defended by Turner who pointed out that Rost was not the first investigator to make a mistake with regard to the cultivation of *Mycobacterium leprae* and most probably would not be the last. Buermann and Gougerot (173) repeated Rost's work with the treatment of leprosy with leprolin and state that in a few cases the material seemed to be of help but in most cases the results were irregular.

Again there ensued a period, lasting until 1909–1910, when no new observations were recorded with regard to cultivation of *Mycobacterium leprae*. Up to this time (1905) no work on cultivation had been generally accepted. The bacillus of leprosy still remained apparently the classical obligative parasite. While no new work on cultivation was reported between 1905 and 1909 there were, as usual, many papers dealing with other phases of the leprosy problem. We find contributions by Hansen (174), who published a paper on his views concerning the fish theory and stated his disbelief in this idea; by Sticker-Bonn (175), who stated a similar disbelief in this theory; by Hamann (176), who brought together a list of papers in the literature on leprosy during the year 1906–07 comprising 86 titles; by Gravagna (177); by Fick (178) (179) who made some further observations on staining *Mycobacterium leprae*, by Woolley (180), who described a method of treating leprosy by injecting phenolized suspensions of *Mycobacterium leprae*; by Unna (181) (182) (183) on staining *Mycobacterium leprae*; by Ashmead (184), who reported treating leprosy with yeast with good results; and by Wherry (185) (186) who presented a study on rat leprosy and observations on acid-fast organisms from rat leprosy and human leprosy in flies caught after feeding on infected animals and human lepers. This author also found the bacillus of rat leprosy in the rat louse. Sugai (187), like Wherry, studied the agglutination of *Mycobacterium leprae* in the serum of lepers, but, unlike Wherry, found that *Mycobacterium leprae* agglutinated in dilutions as high as 1:500 and with most types as high as 1:100 within thirty to forty minutes, though control sera were entirely negative. Others who contributed during this period were Arning and Lewandowsky (188), on staining *Mycobacterium leprae*; Gaucher (189), who stated that serological tests with *Mycobacterium leprae* were of value as an aid in diagnosis; Campana and Carbone (190), who reported the enzymatic digestion of leprosy tissue; Campana (191) (192), in a series of papers, in one of which he advocates cremation of the bodies of lepers; de Buermann and Vaucher (193) (194), who maintained interest in the question of the presence of *Mycobacterium leprae* in the circulating blood of lepers; Lutati (195), who described the granular degeneration of *Mycobacterium leprae*; Silberschmidt (196); Recio (197), who discussed the mode of spread of leprosy and concluded that given a predisposition to the disease the chief avenues of entrance of the infectious agent are through the nasal mucous membranes and by way of the hands, especially in children; Sprecher (198), who suggested the use of the antiformica method to prevent putrefaction of the leprosy bacillus for medico-legal purposes; Düring (199), who commented on hereditary factors in leprosy to which he gave scant importance; Uhlenhuth and Steffenhagen (200), who also employed the antiformin method with leprosy material; Hamann (201), who brought together another list of 124 titles on leprosy in 1909; Boeck (202), who reported finding *Mycobacterium leprae* in the feces where he stated it might remain for a year or more; and Luciano (203). The foregoing list of papers gives at least some idea of the various lines of investigation which were in progress during this period.

This brings us well into the year 1909 and nearly to the end of the period which we have arbitrarily called the "early period" of the bacteriology of leprosy. For perhaps no definite reasons we have concluded to consider in the early bacteriology of this subject the work reported up to about 1918. This date incidentally did not bring us any accepted solution of the problem of cultivation of *Mycobacterium leprae*, and reported investigations continued to stimulate further research in this field.

In 1909 there appeared the observations of Clegg (204) (205) in the Philippines. This investigator reported the cultivation of *Mycobacterium leprae* in symbiosis with the amoeba on the theory that the organism grows by getting its nutrition from the products of tissue cells in the lesions. The amoebae were to provide similar nutritive material. Media for growing the amoebae consisted of 20 grams of agar, 0.3 gram sodium chloride and 0.3 gram of beef extract, with a reaction of one per cent alkaline to phenolpthalein. Plates were poured and material containing the amoebae was spread upon the surface. If symbiotic bacteria were present Clegg stated that the parasite grew in from two to ten days. Leper spleen emulsion containing *Mycobacterium leprae* was then spread over the amoeba culture and incubated for six days at 37°C. By serial sub-culture Clegg states that definite multiplication of acid-fast organisms was demonstrated. Control plates were negative for acid-fasts. The acid-fast rods were short and plump and different from the usual morphology of *Mycobacterium leprae*, according to Clegg, but he rightly points out that nothing was known for certain about the morphology of this organism upon artificial medium. Clegg employed such organisms as the cholera vibrio and *B. typhosus* as symbiotic bacteria in his cultures of the amoebae. Teague, commenting on Clegg's work at the time, stated that he believed the organisms were multiplying. Strong, however, took a more conservative point of view and was only willing to state that he thought it "appeared" that Clegg had been successful in cultivating *Mycobacterium leprae.* Strong further stated that he regarded the possibility of producing satisfactory lesions in animals only remote. In another communication Clegg stated that by heating the symbiotic culture of amoebae, symbiotic bacteria and *Mycobacterium leprae* for one half hour at sixty degrees centrigrade a pure culture of *Mycobacterium leprae* was obtained. Thus isolated, his *Mycobacterium leprae* grew on ordinary laboratory media. In this paper Clegg also stated that inoculation into guinea pigs resulted in lesions macroscopically and microscopically similar to leprosy in human subjects. This organism of Clegg belongs with the group of acid-fast chromogenic bacilli which several investigators have isolated from leprosy material.

During the same year Teague (206) made extracts of leper nodules, spleen, and the skin of cholera patients (as controls) with five per cent glycerin and vaccinated fifty lepers and fifty controls. Most of the reactions were the same in both groups. Wooley (207) had previously used saline extracts as a leprolin.

In 1910 we have the first reports of Duval (208) (209) on the cultivation of *Mycobacterium leprae.* Duval states that his experiments began in 1909 and that the work of Clegg and Sugai (who infected Japanese dancing mice with leprosy material) led him to attempt to confirm their observations and "to attempt further to grow the bacilli directly from the tissues without the aid of symbiotic organisms, and to prove by animal inoculation the identity of the culture." We quote the following summary and conclusions from this author's first paper:

> Pure cultures of an acid-fast bacillus were cultivated upon special media from the human tissues in four cases of leprosy. The nature of the growth, morphological characters and tinctorial properties do not differ for any of

the cultures and correspond closely to the bacilli in the human leprous tubercles.

That the bacillus of leprosy will multiply and continue to do so indefinitely outside of the animal body was first demonstrated by Clegg who cultivated an acid-fast organism from leprosy tissue in the presence of ameba and their symbiotics. Not only have I been able to confirm Clegg's work, but in addition I have succeeded in growing the bacillus in pure culture and in reproducing the disease in the Japanese dancing mouse, thereby establishing its identity. This species of animal acquires the infection in four to six weeks after intraperitoneal or subcutaneous inoculation with either emulsions of fresh leprous tissue or the pure culture of B. *leprae.* Comparatively few bacilli are necessary to infect the mouse; and the mode of inoculation does not seem to make any appreciable difference in respect to the nature and time of development of the lesion.

The experimental lesions are proliferative in character and identical with those in the human subject. Macroscopically they appear as glistening, white nodules which, in the early stages of development, resemble miliary tubercles.

In my experience neither the culture nor the bacilli directly from the human tissues have shown any evidence of multiplication or given rise to lesions when injected into the ordinary laboratory animals such as guinea pigs, rabbits, gray or white mice and rats, although repeated attempts have been made to infect these animals.

B. *leprae* will not only multiply but it will colonize on a plain agar medium seeded with a pure culture of encysted ameba—and upon an agar or banana medium prepared with a 1 percent solution of cystein and tryptophane. Colonization occurs in the form of glistening, white colonies, one to two millimeters in diameter, in from one to two months incubation.

The bacilli in cultures are at all times acid-fast and differ only in morphology from those of the tissues in that they exhibit a greater variation in the distribution of the chromatin and are longer and more distinctly curved.

To prove that the cultures obtained from the human tissues of these four cases are leprosy bacilli and not some other acid-fast species, the following facts are offered: (1) the growth features are distinctive and multiplication takes place only under special conditions of temperature and medium; (2) the complete correspondence in tinctorial properties and similarity in morphology to those in the tissues; (3) the failure to multiply or produce lesions in the common laboratory animals; and (4) the growth of the bacilli and the production of typical leprous lesions in the Japanese dancing mouse.

The successful cultivation of *B. leprae* and the fact that the cultures retain pathogenic properties are of commanding importance in respect to a possible production of an artificial immune serum for combating the infection in man. Work along this interesting line is already in progress in our laboratories.

The entire summary and conclusions of this first paper of Duval have been presented in full to illustrate the convincing nature of his presentation. In the author's other publication of this same year (New Orl. Med. and Surg. Jour.) Duval states that once *B. leprae* start to grow rapidly tryptophane and the amoeba are not essential. Cultures grow well on any neutral or slightly alkaline medium of human or rabbit blood agar and glycerinated serum agars. This organism grown by Duval from leprous tissue belongs also to the group of acid-fast chromogenic cultures to which Clegg's bacillus belongs.

In 1910 still another organism was cultivated from leprosy tissue by Serra (210), who succeeded in cultivating an organism from three out of seven cases. He employed Campana's medium combined with sterile organs of guinea pigs, and subcultures were obtained in glucose agar in seven and eight days in the depths of the tubes, suggesting again an anaerobic form. Animal experiments were negative.

The following year Duval (211) published another paper in which he came to the following conclusions, to quote from his paper:

> In the cultivation of *Bacillus leprae* the initial multiplication outside the body cannot be obtained unless amino-acids are present in the medium. The amino-acids are believed to be essential nutritives for the initial growth of the organisms.
>
> It has been demonstrated that the primary growth of the leprosy bacilli occurs only in the presence of the products of tryptic digestion.
>
> Hence, putrefactive and other bacteria which are capable of splitting nucleo-proteids into their end acid products are, in consequence, of value in the isolation and cultivation of the leprosy bacilli. Amebae are not necessary for securing the primary multiplication of the leprosy bacilli upon artificial media and are detrimental since they feed with avidity upon the bacilli themselves.
>
> Two methods may be employed for recovering in culture *Bacillus leprae* from the tissues. In one (the direct), tryptophane or a mixture of albumen and trypsin are employed with a culture medium; in the other (indirect), bacterial species capable of digesting the albumen constituents of the cul-

ture medium are introduced into the medium. In both, the end result is identical, since they both provide for the presence of the amino-acids in the medium, without which the primary multiplication of the leprosy bacilli cannot be secured.

In the paper from which the conclusions are quoted above, Duval states that, after the initial culture has been started, "growth is luxuriant and reaches its maximum in forty-eight to sixty-four hours." In another section of the paper he states "like the tubercle bacillus they require *abundant* oxygen" (italics ours). Both of these statements will be referred to later on in this review.

In 1912 Duval and Wellman (212) published a paper on a new method of cultivating *Mycobacterium leprae* from the tissues. The new method was based upon the use of mammalian placental tissue extract either in a liquid medium or with glycerinated agar. Placental extract medium was originally employed by Kedrowski in 1901 who grew his diphtheroid from leprosy tissues upon it. In the cultures of Duval and Wellman the growth was profuse and to quote these authors, "not only the initial cultures, but subsequent transplants, in the case of *B. leprae*, flourish on this medium." Some of the cultures were without pigment while others were distinctly chromogenic, according to the authors.

Again, in 1912, Duval and Wellman (213) published a critical study of the organisms cultivated from the lesions of human leprosy with a consideration of their etiological significance. In their summary they state:

> From the leprous lesions two varieties of acid-fast bacilli may be cultivated, one a chromogenic pleomorphic organism which grows readily upon the ordinary laboratory media after it has become accustomed to a saprophytic existence; the other, a moist-growing non-chromogenic bacillus resembling tinctorially the tubercle bacillus, and morphologically the diphtheria bacillus, and multiplying only upon special media. The chromogenic strain, although hard to cultivate at first, subsequently grows profusely and rapidly upon a great variety of foodstuffs, while the non-chromogenic strain is always difficult to cultivate and multiplies very slowly even in generations far removed from the parent stem.
>
> The chromogenic culture may show a wide variation in morphology and its ability to retain the stain when subjected to decolorizing agents. At

times and under certain conditions the individual rods are diphtheroid and non-acid-fast. The nonchromogenic culture is always acid-fast and can be sharply differentiated from chromogenic culture by its growth features.

The authors draw from this work the following conclusions:

(1) From a bacteriological study of 29 cases of leprosy we have isolated an acid-fast bacillus from 22 cases.

(2) A chromogenic strain similar in all essentials to that described by Clegg was recovered from 14 cases.

(3) Eight cases yielded an organism which is markedly different in its character from Clegg's bacillus and which will grow only on specially prepared media and refuses to become chromogenic.

(4) In one case we have isolated a non-acid-fast diphtheroid bacillus corresponding to the organism described by Kedrowski.

(5) We are unable to confirm the work of Rost, Williams, Bayon and others who consider that *B. leprae* is a bacterium of such pleomorphism that it can be recognized as a diphtheroid, a streptothrix and an acid-fast bacillus.

(6) Animal experiments undertaken for the purpose of differentiating the acid-fast organisms and to fix their etiological status are not regarded by us as conclusive.

(7) Serological tests, especially those performed with highly immune sera, have proved of some value and tend to show that Clegg's bacillus of leprosy is not related to the ordinary acid-fast chromogenic saprophytes, and that the non-chromogenic lepra culture of Duval is different both from Clegg's organism and from all other acid-fast bacilli.

(8) The rôle played by the chromogenic bacillus of Clegg in the production of leprosy is as yet an unsettled question, although we are at present inclined to ascribe to it a minor if not a negligible part.

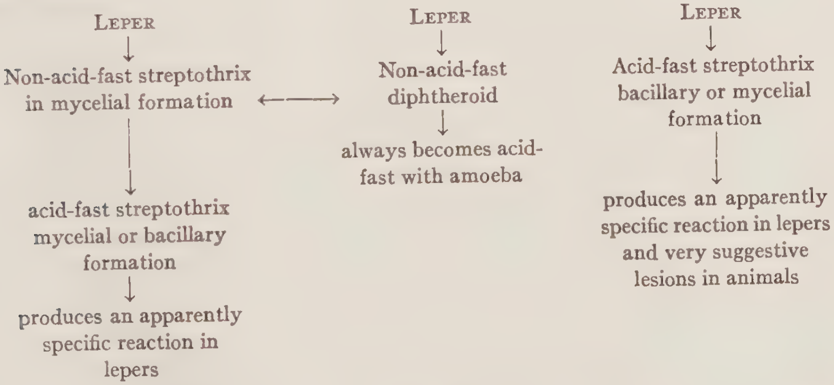
(9) The non-chromogenic strain, while behaving according to most of our notions regarding a pathogenic organism has likewise not up to the present been conclusively proved the cause of leprosy, although we are impressed with the probability of such a rôle being eventually attributed to it and consider that it deserves more serious attention than any organism so far cultivated from the human leprous lesion.

During this same period there were several other papers on the cultivation of the leprosy bacillus. In 1910 Kedrowski (214) (215) reported further work on cultivation as did also Campana (216) and

Küster (217). There was nothing new in these reports. Twort (218) described the use of ground up *B. tuberculosis* added to Dorset's medium. He inoculated nasal discharge and scrapings from lepers and obtained a slow growth of a non-chromogenic acid-fast bacillus which later grew faster, but subcultures on ordinary laboratory media were negative. Currie, Brinkerhoff and Hollman (219) employed Clegg's method and they each confirmed this work independently. The amoeba-cholera lepra cultures were successfully carried through three to ten generations. Williams (220) reported growing a streptothrix and a bacillus on ordinary nutrient broth. The first was non-acid-fast and the bacillus was acid-fast. Both organisms, when injected into lepers, caused general and local reactions. Neither of these organisms, however, were infective for monkeys, rabbits, guinea pigs or rats. McLeod (221) published an interesting review of the state of knowledge of leprosy; this dealt with morphology, staining, classification, cultivation of *Mycobacterium leprae*, inoculation of animals, serology, treatment and mode of spread of the infection. In connection with the question of cultivation of the leprosy bacillus MacLeod stated that "in spite of numerous attempts no medium had been found on which *B. leprae* will invariably grow."

In 1910 Russell (222) reported an interesting case of transmitted leprosy to a human being. The case was a Dutch boy, age 14, who was swimming in a dam on his father's farm and cut his foot on a broken bottle. A native age 20, passing by suggested that he put chewing tobacco over the wound to stop the bleeding. He gave the boy some tobacco that he was chewing and the foot was bandaged. For three years it healed and broke down repeatedly, with discharge of pus. Finally the bone was scraped and the wound healed. This was followed by a rash which the physician thought was probably syphilis and the boy was treated for this condition. Finally he developed an ulcer on the right vocal cord, and the sputum was found to contain *Mycobacterium leprae.* Within six months he developed the facial expression of leprosy and anaesthetic patches. He lived eleven years after the accident. The young native who gave him the tobacco was found to be a leper. There was no trace of the disease in the Dutch boy's ancestry.

In 1911 Williams (223) reported further on his non-acid-fast streptothrix. Concerning his non-acid-fast diphtheroid he states that it and the acid-fast bacillus are phases of the streptothrix. He later converted his non-acid-fast organism into acid-fast diphtheroids when cultivating with the amoeba. He quoted Unna as stating that the leprosy organism includes a large and varied series of forms, which not only include bacilli but other forms differing in morphology and staining reactions, and transitional forms which are inseparable from the growth of leprosy bacilli in the skin. He concludes that the etiology of leprosy is a pleomorphic streptothrix. About the same time Rost (224) reported cures of leprosy by the use of streptothrix vaccines, and Byon (225) discovered an acid-resisting diphtheroid in leprous material which he stated acquired acid-fast properties on being injected into mice or rats. This organism he was able to cultivate in placental-extract agar or horse-serum-metrose agar with the addition of 2 per cent ground up smegma bacilli. The organisms also became acid-fast when cultivated upon Dorset's egg medium. Liston and Williams (226) also reported a streptothrix isolated by them from the spleen of a leper. They state that it resembled the organism originally described by Deycke Pasha and Rost and exhibited many variations in growth, staining, morphology, etc. Then Williams (227) attempted to classify the organisms they found in their work on leprosy as follows:


LEPER
Non-acid-fast streptothrix in mycelial formation
acid-fast streptothrix mycelial or bacillary formation
produces an apparently specific reaction in lepers
LEPER
Non-acid-fast diphtheroid
always becomes acid-fast with amoeba
LEPER
Acid-fast streptothrix bacillary or mycelial formation
produces an apparently specific reaction in lepers and very suggestive lesions in animals

In 1911 Duval, Gurd and Hopkins (228) presented some further studies dealing with immunity in leprosy and in 1912 Duval and Harris (229) and Duval (230) published further papers on the leprosy bacillus and on the status of the bacteriology of human leprosy at that time. Duval and Harris gave the following summary and conclusions in their paper:

> The acid-fast bacillus known in the human leprous lesions as the Hansen organism can be cultivated in vitro under special nutritive conditions. The initial multiplication away from the tissues of the host occurs in the presence of the split-products of animal protein. Although it is reasonable to expect that the sub-culture will in time adapt itself to grow upon other food-stuffs, so far all our attempts in this direction have been unsuccessful. Cultivation experiments indicate that the leprosy organism is unable to attack the whole protein, which would account for the failure of different investigators to propagate the bacillus upon the ordinary laboratory media. The readiness with which the Hansen bacillus multiplies in excised pieces of leprosy nodules that have been hydrolyzed or have undergone autolysis and its inability to grow in the unaltered tissue, or in any medium which does not contain the amino-acids, support this hypothesis.
>
> In the experience of the authors growth of *B. leprae* is extremely slow even under the most favorable artificial conditions, requiring from eight to ten weeks to attain its maximum, at which time it is only comparable in amount to the growth of *B. influenzae*. The accumulation of acid-fast species, apparently does not occur with the Hansen bacillus.
>
> The most efficient method for obtaining the initial growth of *B. leprae* is to transfer bits of the leprous nodule to slanted one per cent alkaline nutrient agar and seed with some one of the proteolytic non-spore bearing bacteria, which in the course of ten days to two weeks at 37°C. digest the protein sufficiently to cause the contained Hansen rods to multiply. It is essential to have the medium alkaline in order to inhibit too profuse a growth of the hydrolyzing organism. In the softened tissue the Hansen bacilli increase steadily and continue to do so in transplants to other media as long as the dissociate products of the host tissue last. The organism used to hydrolize the tissue is subsequently eliminated by heating the culture at 60°C. for thirty minutes (Clegg's method) which does not affect the viability of the Hansen rods. While multiplication takes place readily in the digested host tissue, growth ceases upon other nutrients unless there is added the intermediate products of protein digestion, such as the filtered autolized liver, blood serum, placenta, etc.
>
> Comparative biological studies indicate that the Clegg type of leprosy organism is closely related to the moist growing pigment-producing group of acid-fast saprophytes, while the Levy and Kedrowski cultures, which

are apparently the same, correspond in some respects to avian tubercle and in others to Moeller's smegma bacillus. The Rost and Williams' culture is identical with Grassburger's acid-fast saprophyte, while Karlinski's culture is not to be distinguished from Rabinowitch's butter bacillus.

The serum of rabbits immunized against repeated doses of the Hansen bacilli obtained directly from the uncontaminated human leprous nodule reacts significantly with the "slow-growing" acid-fast culture (Duval) and gives no specific reaction with any of the other so-called lepra cultures.

The experimentally induced lesion affords no reliable means of differentiating acid-fast species other than the tubercle family, since the lesion in all instances has the same general gross appearance. Furthermore, it is not possible to establish an etiological part for any culture isolated from the human leper upon the microscopic character of the experimentally induced lesion since the identical picture may be produced with cultures of Timothy hay, butter, milk, and smegma bacilli.

The specific organism of human leprosy whether in vitro or in vivo is distinctly acid-fast, which feature is as constant for this species as it is for B. tuberculosis. Differences in size and shape of individual bacilli are frequently noted in the same culture; however, these morphological variations, which are often striking in their resemblance to certain forms of the diphtheria group, may be regarded simply as involution types. Non-acid-fast streptothrical and filamentous forms, which have been described as "stages" of the Hansen organism by Kedrowski and others, have not been noted for any culture isolation by us. The specific microörganism of human leprosy is undoubtedly a schizomycete, genus bacillus, and not a trichomycete, genus streptothrix, any more so than the tubercle bacillus.

In 1912 Duval, in the paper referred to above, published a short review of the situation regarding the bacteriology of leprosy and reconfirmed his studies of the two organisms isolated by him. One of these, he states, after cultivation becomes saprophytic and produces pigment; the other grows slowly on special medium and always remains acid-fast. He states that non-acid-fast organisms are occasionally found in external lesions of leprosy. We will omit for the time being the work of this investigator and his colleagues on attempts to produce experimental lesions of leprosy in animals and will consider this subject later under a section devoted to this question.

During this period a number of different phases of the leprosy problem were being investigated and some of these will be referred to briefly at this point. In 1910 Atcherley (231) proposed a definition of the disease of leprosy in which he stated that leprosy is a chronic diathetic disease of gradual onset and irregular course, characterized by an inflammatory fibroid degeneration of the nerve tissue generally, which precedes the deposit of the lepra bacillus, and any other manifestations of the disease. The nerve degeneration, he suggested, is the result or direct consequence of defective pabulum supplied it by the blood, which again is the result of improper food digested by the individual. This definition was advanced by Atcherley, so he states, on the fact that the pathological changes in leprosy bear a remarkable and striking analogy to the corresponding changes in scurvy; and the position of the lepra bacillus is explained by that of the micrococcus, which in scurvy is found in chains along the track of the degenerated arterioles. This is then the basis of the so-called scurvy theory of leprosy. Atcherley states that to speak of an incubation period in leprosy is a myth—that it takes from one to seven years or more after a supposed exposure to develop the disease. He points out, in support of the scurvy theory, that (1) lepers are constantly present in busy seaport towns and the disease does not spread; (2) that although cases of leprosy are common where no contact with lepers can be proved there are none where it can be shown that contact alone caused the disease; (3) that infants never have leprosy; (4) that a child of a leprous mother has no leprosy and if the child develops leprosy he believes it is due to improper feeding and not to contagion; (5) that segregation will fail because it teaches contagion and not good diet; (6) that by habit one living with a leper will take the same food; (7) that spontaneous cures are due to a change in diet; (8) that during a famine in the Philippines the natives ate nothing but clams from a river and many cases of leprosy developed. Atcherley finally concludes that leprosy is a deficiency disease, as is scurvy, and that it has a superimposed infection with *Mycobacterium leprae*—the organisms entering through the gastro-intestinal tract. Other papers of this period were contributed by Crow (232) who discussed the presence of *Mycobacterium leprae* in the circulating blood (as we have seen, an old question of much debate); by Unna (233) on staining; by Marchoux (234) who injected nasal mucous from lepers subcutaneously into white and gray rats and produced abscesses from which he cultivated acid-fast rods on ascitic fluid medium; by Leboeuf (235) who found *Mycobacterium leprae* in the nasal mucous membranes of 159 lepers in a series of 224 examined, or 70.98 per cent positive; by Jeanselme (236) who published a general review concerning certain phases of leprosy and presented a very good bibliography; by Guerra-Coppioli (237) who described *Mycobacterium leprae* in fecal matter; by Davis (238) who reported an interesting case of leprosy contracted in Cape Town; and by Darling (239) who quoted Hansen as stating that the leprosy bacillus must be regarded as an obligative parasite and one which must thrive in the tissues of man. Darling accepts this statement of Hansen (which was made in 1903) as the last word up to that time (1911) on this subject of historic interest. Couret (240) questioned the fish theory of the disease and Bayon (241) called attention to the fact that Sir Patrick Manson was the first to attempt to cultivate the germ of leprosy—that he sealed up the serum of a leper in a little glass tube, placed it inside an egg and put the egg under a hen to hatch. This author reports some interesting new work in which he found he was able to produce nodules and lesions in rabbits by injecting the smegma bacillus and such other acid-fast germs as human and avian strains of *B. tuberculosis*, as well as killed acid-fast organisms. He was unable to produce anything similar to leprosy with Duval's organism and favors the streptothrix as the cause of leprosy; in another publication Bayon (242) states that Kedrowski's organisms are like those described by Hansen and that he also isolated a similar germ. He further states that human and rat leprosy are identical or closely allied diseases, pointing out that it had been reported by Dean that the rat leprosy germ is agglutinated by the serum from human lepers.

A further survey of the literature of this period gives additional evidence of the great interest which existed in the leprosy problem. In 1911 Much (243) reported that the sera of lepers reacted with other acid-fast organisms as well as with *Mycobacterium leprae*; Babes published a review on cultivation work; Alderson (245) reviewed the work of Brinkerhoff, Currie and Hollman in Hawaii (already commented on above) and its significance; Abraham (246) published an additional review on cultivation of *Mycobacterium leprae;* Hutchinson (247) again expressed his belief in the dietetic theory of leprosy, especially the element of badly cured fish; Ehlers, Bourret and

With (248) examined several insects and concluded that the presence of the leprosy bacillus in their bodies is rare; Gurd (249) published a paper on the cytology of leprosy; Hodara (250) reported a similar study dealing with the nerve lesions; Montesanto (251) described a study of the effects of salvarsan on *Mycobacterium leprae* which was inconclusive because of lack of suitable cultivation methods; Terbinsky (252) and Unna (253) had a friendly controversy over staining methods used for *Mycobacterium leprae;* Hansen (254) discussed again the question of heredity in leprosy; Sugai and Menobe (255) reported a histological study of the placenta in both tuberculosis and leprosy patients; Sorel (256) studied the analogy between rat and human leprosy and concluded that in each the infection may remain latent and never produce symptoms of the disease; Smith and Rivas (257) employed trypsinized culture media and reported six successful transplants of *Mycobacterium leprae*; Nakano (258); Paldrock (259) added two papers to the literature on the presence of *Mycobacterium leprae* in the circulating blood of lepers; Leboeuf (260) described enormous numbers of *Mycobacterium leprae* in *Musca domestica*; Lagane (261) added another report on the presence of acid-fast bacilli in the urine of lepers; and Kritschewsky and Bierger (262) brought evidence to favor Kedrowski's culture of *Mycobacterium leprae*, in contrast with that of Duval, on the basis of the Bordet-Gengou reaction.

The group of papers quoted above illustrated the varied interests which were manifested in the leprosy problem during the period covered, now some two decades ago. During the next seven or eight years there were many contributions to the literature dealing with the cultivation of organisms from leprosy tissue. In 1912 Currie, Clegg and Hollmann (263) reviewed the literature on cultivation of *Mycobacterium leprae* and stated their belief in the organism of Clegg. The same year Bayon (264) made a comparative study of the leprosy cultures of Clegg, Duval, Kedrowski, Rost and Williams and he concluded that only Kedrowski's organism, and those similar to his own, have produced leprous lesions in animals and he stated that Duval's and Rost's strains did not, in his experience, cause any lesions when injected into animals. Bayon was of the opinion that the organisms described by these two workers show in their cultural behavior the characteristics of saprophytic organisms and he stated the same also applied to Clegg's culture. During the same year two other papers, not already referred to, are recorded by Duval (265) (266). Nothing new was described in these two papers. During this same year Zinsser and Carey (267) published their work on the cultivation of the organism of rat leprosy in young rat serum and young rat spleen tissue and demonstrated that the organisms grew and multiplied in some of their cultures but apparently never outside of the tissue cells. In 1914–15 other papers came from Duval (268) (269) in which the writer stated that the real organism of leprosy is a bacillus and that it is an acid-fast organism. He pointed out that Kedrowski had in a fifteen years' experience only been able to isolate from two cases what he, Kedrowski, regarded as the germ of leprosy, and further that Bayon had been unable to recover this organism from a second case. In connection with rat leprosy it may be mentioned also that in 1912 Hollmann (270) had reported cultivation of organisms from rat leprosy with Clegg's method and further stated that acid-fasts were demonstrated in the tissues of white rats following inoculation of his cultures. Guinea pigs were negative.

In 1913 Reenstierna (271) isolated organisms[2] from leprosy tissue and blood with which he attempted to infect animals. This work will be referred to later on in this review. Of special interest at this time were papers by Fraser and Fletcher (272) and Fraser (273). These investigators *carefully* removed leprous nodules from 32 lepers in such a way as to prevent contamination and made 373 inoculations on various culture media, including placental agar. They reported that although their leprosy material was swarming with acid-fast organisms they failed even in a single instance to demonstrate any growth or multiplication of the organism and they stated their belief that previous workers who depended upon microscopical evidence of growth must have failed to observe the bacterial richness of the material employed for inoculation. These investigators further emphasize that they cannot comprehend how it is possible to state that in a case where no macroscopic growth is apparent an increase of organisms, recognizable only by the microscope, has occurred. Their cultures were under observation for periods as long as nine months. Fraser states that "inconsistency and pleomorphism are the outstanding features of the recent publications on the subject of leprosy." In another communication two years later Fraser and Fletcher (274) emphasize clearly and forcibly the need for the careful excision of leprous tissue to be used for cultivation purposes. In the work reported in this paper these authors reflected the skin from the nodules (as emphasized by McKinley and Soule in their recent work) and excised the nodules free from contamination. Fraser and Fletcher point out that this is not so simple and call attention to the fact that in their early cultures with such material they cultured diphtheroids in one or two tubes out of twenty. They regarded these organisms as contaminations. They state that all their culture work (on 52 non-ulcerating cases) was negative with such careful technique. None of the mediums advocated gave any positive results and they conclude that there is no evidence that Kedrowski's organism is the true *Mycobacterium leprae.* (These studies of Fraser and Fletcher are very significant in relation to the work of the writer with Soule as will be pointed out in more detail later on.) In 1914 Wolbach and Honeij (275) considered the diphtheroid bacilli in relation to the leprosy problem and in particular to the presence of diphtheroids in normal and pathological tissues. This paper was followed by a second by these same investigators (276) in which they presented a most excellent critical review of the bacteriology of both human and rat leprosy. This paper will be referred to rather extensively in another section of this review since much of what Wolbach and Honeij stated in 1914 regarding this problem remains true twenty years later.

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[2] His blood culture consisted of very long non-acid-fast chains which have later shown acid-fastness. This organism is similar to one later cultivated from the blood by Walker in Honolulu in 1929.

In 1914 Kendall, Day and Walker (277) studied the metabolism of several of the acid-fast organisms including *Mycobacterium leprae*, the grass bacillus and the smegma bacillus, and they concluded that *Mycobacterium leprae* is different from the others in this regard. The same year Johnston (278) reported 28 cultivations of organisms from leprous material of which 20 were diphtheroids, five were other rods and three were streptothrix varieties. Animal inoculations, except in the case of one guinea pig, were negative. Johnston concluded that he was convinced that *Mycobacterium leprae* represents only an acid-fast stage in the life cycle of a markedly pleomorphic streptothrix. In a later paper Johnston (279) classified the various forms of

Mycobacterium leprae as (*a*) the classical type; (*b*) fragmentary or degenerative type (including those with coarse granules and those with fine granules); (*c*) the solid type (including the long form and the short form), and (*d*) the nocardial or streptothrix type. During 1914 we also find an interesting paper by McCoy (280) in which 83 specimens of leprous material were employed for cultivation experiments. One specimen came from a case of the anaesthetic type of the disease but no positive cultures were obtained. Eighteen cases were of the mixed type and three positive cultures were obtained while of 64 cases of the nodular type McCoy obtained eight positive cultures. Nine of the organisms isolated by McCoy showed various shades of yellow and grew freely on artificial media. Two of the eleven cultures grew very slowly on plain or glycerin agar, producing only the slightest suggestion of a yellowish tinge even in old cultures. However, these two strains grew luxuriantly on plain agar to which one per cent of glucose was added. None of the organisms were pathogenic for mice, rats, rabbits, guinea pigs or *Macacus rhesus* monkeys. McCoy stated that it was useless in his experience to make transfers of cultures unless coccoid forms of the organism are present since he felt they are the first evidence of growth. When in clumps, however, no matter what their number, in his opinion one cannot feel certain of growth. McCoy reported that he made transplants two to three days to a week or ten days and on several occasions only one of eight or more tubes would show growth, illustrating how large an element of chance there is in this type of work. His cultures thrived for four or five generations and then disappeared. On a number of occasions what appeared to be a pure culture of an acid-fast organism on amoeba agar turned out to be free, or almost free, of acid-fasts. One culture of McCoy's was a pure acid-fast on amoeba agar but persistently showed large proportions of non-acid-fasts on plain and glycerin agar. Transferred back to amoeba agar, the acid-fasts reappeared.

Rost (281) again in 1914 restated his belief in the leprosy streptothrix. In 1915 Fraser and Fletcher (282) presented further arguments to support their conclusions that the acid-fast bacillus of Kedrowski is not *Mycobacterium leprae.* Bayon (283) described the bacillary deposits he obtained in rabbits with organisms from leprosy nodules and with Kedrowski's cultures. Bayon stated that it is impossible to expect skin lesions in the experimental animal—that only discrete deposits of organisms should be expected in the organs. He pointed out that hundreds of negative observations should not invalidate the proof positive of a single successful inoculation in an animal. In a further paper during the same year Bayon (284) published a comprehensive review of the subject of leprosy and again stated his belief in Kedrowski's culture as the true bacillus of leprosy. At this time Stanziale (285) reported the cultivation of *Mycobacterium leprae* on glycerin agar with and without milk sugar and stated that he obtained beautiful heavy cultures. The year following Harris and Lanford (286) published their work on the agglutination reaction of a number of acid-fast organisms with sera from human cases of leprosy and from experimental animals. They concluded that, "until some further refinement in these procedures is devised but little reliability can be placed upon this type of test as a means of identification of any culture isolated from the lesion of leprosy as the bacillus of Hansen."

During the period of several years for which work on cultivation has just been described there was, of course, much activity in other phases of the leprosy problem. Some of these reports will be mentioned in passing in order to present the broad interest in leprosy which was still being manifested in many phases of the problem. As usual there was great interest abroad concerning the question of the presence of *Mycobacterium leprae* in the blood stream. Papers dealing with this subject were presented by Marchoux (287) who stated that *Mycobacterium leprae* is found in the macrophages and exceptionally in the polymorphonuclear cells of the blood; by Crow (288) who stated that *Mycobacterium leprae* may be found in the circulating blood in about 80 per cent of cases; by Rabinowitsch (289), Lagane (290) and Beurmann and Gougerot (291) all of whom described *Mycobacterium leprae* in the blood stream, the latter investigators also describing *Mycobacterium leprae* in kidney lesions; by Rivas and Smith (292) and Rivas (293) (294) (295) who emphasized the bilateral distribution of the infection in relation to its bacteremic nature; by Honeij (296) who demonstrated *Mycobacterium leprae* in the blood and called attention to the fact that this observation warrants the assumption that insect transmission of the disease is a possibility; by Alfonseca (297) who reported *Mycobacterium leprae* in a placenta as well as in various secretions of the body; and by Hollmann (298) who studied the blood of 22 lepers and found acid-fast organisms in six of these. In two of these cases only a single organism was to be found. In one case of nodular type, and during leprous fever and the period of new nodule formation, many acid-fasts were demonstrable in the circulating blood, but after this acute attack no organisms could be found. Iyengar (299) found *Mycobacterium leprae* in the blood of seven out of forty cases examined. Papers on the relation of rat and human leprosy were published by Bayon (300) and Marchoux (301); on insect transmission by Verteuil (302) who concluded that blood sucking insects transmit the disease; by Aragas (303) who considered the possibility of mosquito transmission; by Azevêdo (304) who published a review of the same question but added no new information and by Cumston (305) who discussed the various modes of spread of the infection including that by way of insects, particularly *demodex* as suggested by Borrel. Merian (306) published an interesting paper on the appearance of *Mycobacterium leprae* in a cowpox pustule following vaccination.

During this same period general papers such as reviews, on treatment, distribution of the organism in various tissues of the body, new pathology of the disease—such as in bone, or statistical reports on the disease in different parts of the world, as in Norway or in Rio de Janeiro, were published (to mention only a few authors) by Boeck (307); Becares (308); Thompson (309); Deycke (310); Rost (311) on the clinical use of his streptothrix vaccine; Verteuil (312) who employed radium varnish to treat leprous nodules and changed bacillary forms of the organism to granular forms; Reenstierna (313); Leboeuf and Javelly (314) who searched in the cervical ganglia for *Mycobacterium leprae* and found them in one case in a series of ten cases examined; Kraus, Hofer and Ishiwara (315) who attempted differentiation of *Mycobacterium leprae* by bacteriolysis with both exudate and serum; Hopkins (316); Matas (317); Couvy (318); Arning (319); Azevêdo (320); Gwyther (321) who raised the question of a primary lesion in leprosy; Chipman (322) who reported that the combination of chaulmoogra oil and resorcin was the best available treatment for leprosy for the time; Candido (323); Kryle (324), Sordelli and Fischer (325) (326); Aiyar (327); Römer (328); Gomez (329); Honeij (330) who described early changes in the epiphysis at the distal ends of the bones and a decrease in the circumference of the distal phalanges of the little finger in cases of leprosy and stated that the bone changes ranged from slight thinning to early atrophy and total absorption; Johnston (331); Joltrain (332) who reported a case of leprosy in an individual who had left Senegal only two years before; Voisins (333); Solari (334); Rudolph (335); Almeida (336); Terra (337) and others. These various papers kept alive the interest in the leprosy problem in various parts of the world and are mentioned briefly only as a background to the work which was in progress in the field of bacteriology of the disease. This brings us to the close of the early period on cultivation experiments with *Mycobacterium leprae* and a critical evaluation of this work will appear in another section of this review later on.

Chemistry of certain acid-fast bacteria obtained from lepers

Considering the period of investigation up to around 1918 which we have reviewed thus far, we find that very little work was carried out on the chemistry of the organism. The first clues to knowledge concerning the chemistry of Hansen's organism were discovered in the study of the staining reactions of the bacillus. Such early papers as those of Unna (82) (72) led the way and it was early recognized that *Mycobacterium leprae* and *M. tuberculosis* were similar in their content of fatty substances and differed from most other organisms in this respect. The acid-fast nature of these two organisms was also known fairly early after their discovery. Later, attempts were made to utilize the neutral fat designated as "nastin" in the treatment of leprosy, but this fad did not last long and the method soon fell into disrepute. In 1911 Much (243) pointed out that *M. tuberculosis* contains albuminous substances, neutral fats in combination with fatty alcohol, a mixture of fatty acids and lipoids. He states that *Mycobacterium leprae* contains the same substances but probably not the poisonous substance which is present in *M. tuberculosis*. That there is a relationship between these organisms he pointed out in an experiment in which tubercle-immunized goats were injected with *Mycobacterium leprae*. The injection was followed by extensive leprous alterations.

During the same year Gurd and Denis (338) studied the chemistry of Duval's strain of supposed *Mycobacterium leprae* and concluded that the protein portion of this organism represents practically the whole of the toxic element. In 1907 Deycke Pasha and Reschad Bey (339) described "nastin," previously referred to, and stated that they originally obtained good results in treating leprosy with the streptothrix vaccine; they found later that this result was due to the fat content of the organism, hence their use and advocation of "nastin" as a therapeutic agent in the disease. In 1910 Unna (340) made further investigation of the fat content of *Mycobacterium leprae* by staining reactions following various methods of fixation with alcohol, acetone, chloroform and different concentrations of alkali. Unna (341) also suggested a method of staining to determine whether the organisms were living or dead. If the organisms stained yellow or brown with safranin and were acid-resistant they were considered, in his opinion, dead, and, of course, without virulence.

Naturally during the period under discussion there were many limitations imposed upon investigators in the study of the chemistry of *Mycobacterium leprae*. It must be recalled that there were no acceptable cultures of the organism of leprosy and even if there had been, the chemical methods of micro-analysis at that time were extremely limited. Since we are attempting to develop the story of the study of the etiology of leprosy in somewhat of a chronological order it may perhaps be best to discuss the more modern chemical work on the various strains of *Mycobacterium leprae* in a later section of this review even though most of these cultures had been isolated twenty years or more previously. We will return then to this discussion later.

Animal experimentation and results

From the discovery of *Mycobacterium leprae* by Hansen through the several decades up to 1917 there were many attempts to transmit leprosy to experimental animals by the injection of leprous material in the form of tissue emulsions containing large numbers of acid-fast organisms (presumably *Mycobacterium leprae*) or supposed cultures of the organism. In this section we will review most of the important work reported during this period, or up to the section which we have arbitrarily designated the newer knowledge of the bacteriology of this disease.

In 1932 Soule and McKinley (342) pointed out that attempts at the experimental transmission of leprosy to man had been very discouraging, and that there was perhaps no case of experimental human transmission of the disease on record (out of 145 human inoculations) which could be accepted as a proven fact (with the possible exception of the Arning case in Honolulu). Though most of the experimental animal transmission work was recalled in this report, the conclusion seemed to be inevitable that no true leprosy had as yet been reproduced experimentally in the many species of animals which have been tried over the many decades since Hansen observed *Mycobacterium leprae* in the tissues of lepers. It is a rather striking fact then that the transmission of this disease to either man or to animals has remained extremely questionable, if not definitely denied by most leprologists. Certainly nothing accepted frankly as leprosy in an animal (leaving aside for the moment the question of rat leprosy which most investigators feel is a different disease from leprosy in man) has ever been produced according to the minds of the most critical observers in this field of work.

The attempts to transmit leprosy to lower animals began very early after the discovery of *Mycobacterium leprae* by Hansen. Indeed Hansen himself was one of the first to attempt to reproduce leprosy in animals. In 1882 Hansen (27) reported that inoculation of rabbits and cats by him were negative and he called attention to the negative results of Köbner (28) with monkeys and fish. Neisser's (26) early animal experiments were also negative in so far as "real leprosy" is concerned, although suspicious nodules were produced by him in both dogs and rabbits. Hansen (22) also inoculated monkeys without success. These early papers on animal inoculations apparently did not stimulate much effort along this particular line of investigation, yet one finds scattered references here and there regarding attempted animal transmission, which are mostly negative in character. Azzarello (139) reported negative results. Klitin (170) (171), however, inoculated both guinea pigs and rabbits with cultures of diphtheroids obtained from leprosy material and reported the successful production of lesions with the recovery later of short granular organisms. These reports are not very convincing. Among the very early papers dealing with this subject were those of Bordoni-Uffreduzi (62), Lévy (99) and Czaplewsky (101), all of whom employed supposed cultures of

Mycobacterium leprae in the inoculation of guinea pigs, rabbits and mice with negative results. In 1900–01 Kedrowski (135) utilized his cultures of *Mycobacterium leprae* grown upon placenta agar and glycerin agar and he reported that he was able to produce in rabbits granulomas containing acid-fast bacilli eight months following inoculations. In 1905 Nicolle (343) described experimental lesions of leprosy in monkeys. Nicolle employed bonnet monkeys and injected saline emulsions of leprous tissue containing *Mycobacterium leprae* in several places. The inoculations were made by various methods, viz., by scarification (temporal-frontal region), by friction (conjunctival and nasal mucous membranes) and by subcutaneous injection (over left eye and in eyebrow). Four days after the inoculations no signs of the injections remained. On the sixty-second day a subcutaneous nodule appeared and three days later there was a marked extension of the lesion with the skin adherent. A week to ten days later the lesion, which had remained unaltered, was removed. Section of this lesion showed numerous lymphocytes, mononuclear leucocytes, a trace of caseation and *Mycobacterium leprae,* two or more in cells. Nicolle believed the lesion to be a true leprosy. Three years later Marchoux and Bourret (344) reported "negative" or "doubtful" lesions in chimpanzees following inoculation of leprous material. In 1909 Clegg (204) (205) observed lesions in guinea pigs following the injection of his cultures, which he thought were quite similar to leprosy in human subjects, but which were not convincing enough to be judged positive, as subsequent events proved. A year later Stanziale (345) described the production of an experimental lesion of leprosy by injecting leprosy material into the anterior chamber of the eye of a rabbit. The same year Nicolle and Blaizot (346) injected the *Macacus sinicus* with leprosy material and obtained positive lesions in two animals in sixty-two and sixty-eight days and demonstrated bacilli on the fifty-sixth and thirty-seventh days. They inoculated one of these animals again twenty-three days before the disappearance of the first lesion and after thirteen days a cold abscess appeared which persisted for sixty-three days. In the second animal they produced lesions which persisted for different periods of time ranging from twenty-nine days to one hundred and fifty days. Their work seemed to indicate that repeated inoculations resulted in a diminution of the incubation time and in lesions which persisted for increasing periods of time. Microscopically their lesions appeared like those of human leprosy with *Mycobacterium leprae* present both inside and outside of the cells. Serra (210) also reported work on attempts to produce experimental lesions in guinea pigs, rabbits, dogs and rats utilizing cultures of anaerobic bacilli obtained from leprosy material but his results were negative.

In 1911 Bayon (225) injected his acid-resisting diphtheroid into mice and rats and later he recovered the organism from the glands. The same year Marchoux (234) produced local abscesses under the skin of grey and white rats with nasal mucous from lepers and the abscess material was found to contain acid-resistant bacilli which he was able to cultivate in such media as ascitic fluid. Marchoux, however, was not sure that he was actually dealing with *Mycobacterium leprae*. At this same time Bayon (241) reported the production of experimental lesions in rabbits following the introduction of cultures of *B. smegmatis* around the sciatic nerve. He also found that such lesions could be produced with *B. tuberculosis* (both human and avian) and later he found he could produce such lesions with killed cultures of acid-fast organisms. In 1911 Duval (347) also reported work on the experimental transmission of leprosy to the *Macacus rhesus* and, among other things, this author concluded that "The analgesia of the leprous nodules with which are associated hypersensitive zones in the monkey happened to be a marked feature of the cutaneous lesions of leprosy in man. This fact and that of the occurrence of erythematous patches, thickening and pigmentation of the skin in areas far removed from the inoculated site in the monkey, seem to prove conclusively the spread of the infection from one side to another, and to justify the statement that leprosy is reproducible in the monkey, in which animal many of the clinical and pathological phenomena common also to the human subject may be advantageously studied." This author's work with experimental inoculation of the Japanese dancing mice has been alluded to elsewhere.

In a further paper Duval and Gurd (348) state in their summary and discussion that; "Repeated experiments have proven that few, if any, of the ordinary laboratory and domestic animals are immune against infection by *Bacillus leprae*. As previously reported, the goat, horse, guinea pig, and many cold-blooded animals (Couret) have been found susceptible to invasion by this organism." During the same year Couret (349) reported that the leprosy bacillus 'survives and multiplies in cold-blooded animals, at least at room temperature in a warm climate. . . ." For his experiments Couret employed such animals as tadpoles, frogs, snakes and turtles, and in addition he used fish such as gold fish (*Carassius auratus*) and spots (*Leiostomus Xanthurus*) not to mention a few mullets and croakers. The following year Duval and Couret (350) published a further paper on the production of leprosy in the monkey (*Macacus rhesus*) in which they state that "the production of leprosy in the monkey *proves conclusively* that the acid-fast bacillus cultivated by one of us (Duval) from the human lesion is the Hansen bacillus and not some extraneous saprophyte, and that it is the etiological factor in human leprosy" (italics ours).

Meanwhile further papers dealing with this very interesting question of animal transmission of leprosy, were published by Serra (351) who reported on the production of leprosy lesions in the eye of the rabbit; by Nicolle and Blaizot (352) on lesions produced in the chimpanzee and lower monkeys; by Stanziale (353) (354) who reported again on experimental lesions in the anterior chamber of the rabbit's eye in which he stated multiplication of *Mycobacterium leprae* took place; by Bayon (264) who stated that only Kedrowski's organism is capable of producing lesions in animals and that Duval's and Rost's organisms do not cause leprous lesions, pointing out that the latter two possess a cultural behavior like that of saprophytic organisms, by Hollmann (270) who employed Clegg's organism and demonstrated the organisms in the tissues of rats following inoculation; by Serra (355) who again described the experimental lesions in the eye of the rabbit; by Truffi (356) on the same subject; by Nakano (357) who described experimental lesions of leprosy in the Japanese house rat; by Machow (358) who stated that Kedrowski's culture is little or only slightly pathogenic for mice; by Chirivino (359) on the production of experimental leprosy nodules in the anterior chamber of the eye; by Bayon (360) who reviewed the subject of animal transmission and concluded that the most suitable animals are the rabbit, rat or mouse. Other investigators (to mention only a few) who published at this time were Reenstierna (271); Verrotti (361), who produced leprosy nodules following intraperitoneal injection of leprosy material into rabbits; Bayon (362), (a review); Stanziale (363); and Verrotti (364) who reported on experimental lesions of leprosy in monkeys.

In 1914 Bayon (365) published another review of the question of animal transmission of leprosy. After inoculating over four hundred common laboratory animals he concluded that such animals are rarely infected but when they are their lesions are "identical with those met with in nerve organs of lepers." During the same year Johnston (278) reported negative lesions in guinea pigs, with one exception, following the inoculation of these animals with a culture of a streptothrix isolated from the spleens of two lepers in the Philippines. One guinea pig showed an enlarged liver with nodules from which the organism was recovered. It was also at this time that McCoy (280) reported his culture work and found that none of his cultures were pathogenic for mice, rats, rabbits, guinea pigs or *Macacus rhesus* monkeys. The following year Bayon (283) described the "bacillary deposits" which he produced in rabbits and in this report stated frankly that he felt that skin lesions of leprosy in animals could not be expected but that "partial and incomplete interpretation of hundreds of negative observations cannot invalidate the proof positive of a single successful inoculation." Bayon (284) followed this communication by a further review of the experimental study of leprosy but his conclusions regarding animal transmission were the same as in the paper quoted above. In 1916 Kryle (324) introduced leprosy tissue into three *rhesus* monkeys and described the appearance of nodular lesions which appeared in eighteen to twenty-two days, similar, in point of incubation time at least, to those of McKinley and Soule (5) of many years later. About this time another paper on the production of leprosy lesions in the anterior chamber of the eye of the rabbit was published by Lutati (366).

From these many reports on transmission of leprosy to lower animals it is readily apparent that down through the years there was a wide divergence of opinion as to the susceptibility of any of the animals studied, to infection with leprosy. Considering the work reported up to this point, we cannot help but be left with a feeling that there is indeed grave doubt whether any of these investigators actually produced anything like real or true leprosy in any of the animals with which they experimented. Some of the reports, as we have seen, were quite positive and dogmatic in their claims. Others were conservative and less certain. The controversy continued over several decades, with several investigators advancing their claims for the actual cultivation of the true Hansen bacillus and endeavoring to prove their claims by the experimental production of leprosy in the laboratory animal, either with cultures, or with leprosy tissue from the patient. It seems clear that certainly up to 1918 the work of no individual on the cultivation of *Mycobacterium leprae* could be accepted as proven fact or as final in any sense. Furthermore, the work of no investigator who claimed to have produced the disease leprosy experimentally in a lower animal (or even in man for that matter) was generally accepted. The period which we have designated that of the early bacteriology of the disease must therefore be regarded as one of doubt and uncertainty. Before taking up the next period, which we have designated the period of the newer knowledge of the bacteriology of leprosy, it may be profitable to evaluate the knowledge of Hansen's bacillus gained during the earlier period, as a preface to a discussion of the more recent work.

NEWER KNOWLEDGE OF THE BACTERIOLOGY OF LEPROSY

Critical evaluation of previous knowledge of Hansen's bacillus

In reading over the previous pages of this review the author finds little to warrant the opinion that up to 1918 there existed satisfactory evidence of a single critical experiment upon which to base the establishment of the etiological agent of leprosy, as judged by Koch's postulates. The publications reviewed cover a period of over four decades following Hansen's first description of the bacillus which bears his name. In many ways it is amazing that after forty-five years of work (up to 1918) on this problem by so many investigators the results remain in such an unsatisfactory state. So many claims were made for the successful cultivation of the actual organism of leprosy, and for the transmission of the disease to several species of animals, that a conservative thinker is tempted to view all claims or assertions regarding success in either of these two fields with something more than the proverbial grain of salt. Indeed, as late as 1925, Rogers and Muir (367) in their chapter on the etiology of this disease state:

> Although more than fifty years have passed since the discovery of the lepra bacillus by Hansen, we have no certain proof that this organism has ever been cultivated *in vitro*. No other organism has ever resisted the efforts of bacteriologists so long.

Even five years after this, in 1930, Bulloch (368) stated in his review of this subject:

> In spite of very extended efforts by bacteriologists in all countries it appears to be still the fact that the bacillus has not been successfully cultivated *in vitro*, nor has the disease been conveyed experimentally to animals or even to man.

Again, in 1932, Soule and McKinley (342), after reviewing the literature carefully and attempting properly and conservatively to evaluate their own investigations with this disease, stated:

> Since the discovery by Hansen in 1874 of small rods lying within the "lepra cells" this organism has been generally accepted as the cause of the affection, and yet of the many reports on the cultivation of Hansen's bacillus obtained from typical lesions of the disease there is none that has been accepted as establishing proof of the actual cultivation *in vitro* of *B. leprae*.

We have then up to a very recent date a situation regarding the etiology of leprosy which is, to say the least, a most baffling one and a vast literature characterized by claims and counterclaims, none or few of which have been finally accepted. Let us therefore look back over the terrain and attempt to determine, if possible, what can be established as authentic fact from the mass of data which have been reported.

Most of the various forms of organisms which have been isolated from leprosy tissue had already been described before 1918. These include diphtheroids, chromogenic acid-fasts, non-chromogenic acid-fasts, anaërobic bacilli and actinomyces. It is quite apparent at the outset that not all of these different organisms can possibly be the cause of leprosy even though positive claims have been made for each of these forms, not once but repeatedly in most instances. Furthermore, attempts to produce experimental lesions with most of these forms have been reported and, in the opinion of several authors, with definite success. It is rather amazing with what frequency such claims have been made and upon, in many instances, totally inadequate experimental data. We venture the assertion at this point that to date no investigator has succeeded in producing leprosy in any experimental animal or in man with absolute certainty. In making such a statement we do so advisedly though it must be admitted that nobody knows what human leprosy produced in an experimental animal should look like or of precisely what it should consist. If we expect to produce in the experimental animal the precise picture of leprosy as we know it in man, then possibly the experimental disease in laboratory animals may never be established. If, on the other hand, we are willing to accept local destructive and progressive lesions produced in animals as a result of inoculation with leprosy material then there is still hope—indeed more than mere hope, as will be pointed out later.

In a previous section of this review we called attention to an interesting paper by Wolbach and Honeij (276) in which these authors presented, in 1914, a critical review of the bacteriology of leprosy up to that time. The conclusions arrived at by these authors are so nearly true today, in our opinion, that we wish to quote them at some length at this point. Wolbach and Honeij state:

> To draw conclusions from a review of this sort is very difficult. Indeed, any conclusions must necessarily be speculative, for a new technic and a few clear-cut facts can completely change the whole aspect of the subject. It is advisable, however, to discuss the facts accumulated with the hope of defining more clearly the problems still to be solved in the bacteriology of leprosy. First of all we must conclude that at least two, the diphtheroid and pigmented acid-fast, and possibly all four varieties of the bacilli are commonly found in leprosy tissue. The diphtheroid organisms have been found in all parts of the world; the pigmented acid-fast have been found most often in the Philippines and Louisiana, independently and by competent bacteriologists. The non-pigmented acid-fast anaërobic bacilli perhaps have not been found often enough to have special importance. The prevailing opinions as to the nature of the leprosy bacillus, however, force us to regard the few isolations of acid-fast, non-pigmented aërobic cultures as of extreme importance.

Granting the association of several different bacilli with leprosy there are two possible explanations. Perhaps the more plausible is that the organisms gain entrance through open lesions and are carried to various parts of the body where they remain quiescent; it is difficult, however, to account for their presence in early lesions, whence several investigators have obtained each sort. More difficult to believe is it that these organisms are in actual symbiosis and in this relationship are responsible for the disease, leprosy. In favor of the first explanation is that diphtheroid bacilli and pigmented acid-fast bacilli are fairly generally distributed in nature. The former are frequently found on the surfaces of ulcerations, in skin lesions of all sorts, and upon normal mucous membranes. The latter, in the form of the smegma bacillus, are found on the surface of the body, and as the long list of acid-fast organisms now under cultivation testify, free in nature. However, diphtheroids cultivated from leprosy exhibit certain differences from those encountered from other sources, chief of which are partial resistance to acids after staining with carbol fuchsin, difficulty of staining with ordinary aniline dyes and more exacting growth requirements. A research into the nature of the bacteria infecting open lesions in leprosy is clearly indicated to help solve this point.

In connection with the pigmented acid-fast bacilli it must be insisted that the very carefully recorded experiments of Clegg and his associates and Duval and his associates admit of no other conclusions than that these organisms also come from leprosy tissue. Whatever their significance may be, the nature of the organisms, their free growth at ordinary temperatures and upon ordinary media, do not accord with our ideas of a parasite so highly specialized as the leprosy bacillus must be. It is difficult to understand why these cultures are so difficult to obtain in the first generation and so easy to maintain afterwards.

The possibility that the partially acid-fast diphtheroids may be converted into completely acid-fast bacilli must be taken into serious consideration, particularly in the light of Bayon's, Williams' and Kedrowski's recent publications, and because of the well known variation in staining reactions exhibited by leprosy bacilli in tissues (Babes, loc. cit.). The difficulty of obtaining leprosy bacilli in tissues stained with carbol fuchsin, followed by decolorizing agents and the ease with which they are stained with basic aniline dyes, for instance Unna's polychrome blue, are too well known to need comment. Unna believes that the bacilli that take the counterstain are dead, chiefly because they are more numerous in old lesions, but this is by no means proved. We do know that great variation in the resistance to acid decolorizing agents is shown by the tubercle bacillus after many years of cultivation. A fair review of this subject has been written by W. B. Wherry. His own work was done with a tubercle bacillus culture, which was brought from Koch's laboratory in 1888. He has determined some of the conditions by which the cultures could be made either acid-fast or non-acid-fast at will. Growth under conditions unfavorable to the synthesis of fats will make the cultures non-acid-fast. It is interesting to note that the morphological variation was extreme, from coccoid bodies to short or long, thick or thin, straight or curved rods, which were or were not acid-fast, according to the conditions of growth. Similar variations in morphology of tubercle bacilli from human and bovine sources, produced by change of medium, are recorded by Wolbach and Ernst. These few facts indicate the possible claims for consideration of a theory, that, during the long residence in the body, the leprosy bacillus may develop strains exhibiting marked morphological and tinctorial differences, because of the variety of conditions to which they are subject, according to the character of the lesion and its location.

The value of animal experiments in the attempt to show specificity of organisms from leprosy is very questionable at the present time. The instances of successful inoculation of animals with leprosy bacilli direct from human tissues are few and not wholly beyond criticism. Sugai and Duval seem to have produced lesions in dancing mice by means of injections of leprosy tissues. Nicolle and Blaizot give fairly conclusive evidence of having produced slight lesions in *Macacus rhesus* and *Macacus sinicus* by the injection of suspensions of bacilli from man. Stanziale seems unquestionably to have produced active lesions in the anterior chamber of the eyes of rabbits, using tissue from very early leprosy nodules for inoculation. These few instances seem to be the only positive experiments recorded, although numerous workers have produced local abscesses in which they thought multiplication of leprosy bacilli had taken place. On the other hand, inoculation experiments with cultivated organisms have been rather more successful. The pigmented acid-fast cultures isolated by Clegg and Duval have given positive results with guinea-pigs, dancing mice, rabbits, white rats and monkeys. Regarding Kedrowski's experiments with the diphtheroid bacillus we can only call attention to the pertinent criticism of Babes. In general, all attempts to produce lesions with diphtheroid cultures from leprosy have failed. It is quite certain that animal inoculations up to the present time have yielded no evidence of value. Duval finds in his inability to produce lesions with non-pigmented acid-fast organisms an argument in favor of this culture being the true leprosy bacillus, in that the results are at least comparable with those obtained by injections of bacilli obtained directly from human tissues.

It is our studied opinion that the above discussion offered by Wolbach and Honeij is very sound. It is interesting to note that twenty years ago these authors pointed out that the prevailing opinions as to the nature of the leprosy bacillus at that time forced them to regard the few isolations of acid-fast, non-pigmented aërobic cultures as of extreme importance. These organisms are still regarded as of unusual importance in 1934. In speaking of the cultures of Clegg and Duval, viz., the pigmented acid-fast bacilli which both of these investigators had isolated, Wolbach and Honeij state definitely that such organisms do not accord with their ideas of a parasite so highly specialized as the leprosy bacillus must be and they point out that they cannot understand why these cultures are so difficult to obtain in the first generation and so easy to maintain afterwards. That these opinions were sound in 1914 and are regarded as sound even in 1934 is evidenced by the fact that as late as 1930 neither Clegg's nor Duval's organisms were accepted as the true germ of leprosy. The diphtheroids are even easier to dispose of as these authors have pointed out. While there have been but few references to anaërobic forms these have possibly never been considered seriously as related to leprosy. During the past several decades in the history of bacteriology we have learned something concerning bacterial tissue flora. We know, for example, that bacillary forms are commonly found in lymph nodes, and spirochetes have been described in tumor tissues even when such tissues have been well capsulated and protected from surrounding tissues. Naturally the only inference is that various organisms are from time to time gaining access to tissues within the body through the blood and lymph, and that these same organisms are for the most part of such low virulence that they produce no actual infection and are only discovered when such tissues are examined for their presence microscopically or by culture. If this is true in closed tissue, and the evidence is most certain in this respect, then what might we expect in the way of contaminating and saprophytic organisms in open lesions, such as ulcerating lesions? As Wolbach and Honeij pointed out in 1914, such organisms as diphtheroids are frequently found on the surfaces of ulcerations, in skin lesions of all sorts and upon normal mucous membranes. In view of these observations, which are today common knowledge to all trained pathologists and bacteriologists, we are forced to conclude that much of the early work on the bacteriology of leprosy is confusing because many workers were unwittingly working with contaminated materials and hope was, to some extent at least, paramount to good judgment when evaluation of experimental data was presented. We do not insist that all workers were concerned with contaminated materials but in many instances it is the only explanation which will elucidate the bizarre results which have been reported. In this regard may we recall once more than in 1915 Fraser and Fletcher (274) emphasized the importance of removing leprosy tissue with strict aseptic technique for cultivation work. When this was done these workers were unable to cultivate any organisms whatsoever from leprosy tissue. We regard the experiments of these investigators as of decided significance in the light of our own work with Soule, which will be described later. It is an all too common practice to remove leprosy nodules from patients by literally "shaving" them off. We have received such material in our own laboratory in which skin and subcutaneous tissue (the nodule intact) constituted the specimen, the skin bearing its usual abundance of hair. Manifestly such material should not be employed for cultivation work though it seems lamentably to be the fact that it frequently has been so employed in the past.

Thus far we have considered only the diphtheroids, the pigmented acid-fasts and the anaërobes. What of the non-pigmented acid-fasts? These, as Park and Williams (369) have recently stated, constitute the modern conception of what the true leprosy organism should really be. This also appears to have been the general feeling in the matter twenty years ago. The non-pigmented acid-fasts of such workers as Weil, Karlinsky, Marchoux, Twort and Duval and Wellman (leaving aside Kedrowski's acid-fast diphtheroid) were all non-pathogenic for animals. The organism isolated by Duval and Wellman (213) they themselves admitted was not conclusively proved the cause of leprosy. In connection with this organism these investigators stated that it grew well on amino-acid medium but would not multiply on ordinary laboratory foodstuff when first isolated though later amino-acids were not essential for growth. The organisms isolated by McCoy (280) should also be mentioned, particularly the two strains which grew slowly on glycerin agar with only a slight tinge of yellow. It is noteworthy, however, that these strains grew luxuriantly on plain agar to which glucose was added. The organisms were not pathogenic for several species of animals inoculated and most probably are not significant as far as the true germ of leprosy is concerned though they certainly must be considered along with other organisms of their type.

After having considered the most important types of organisms which were described up to 1918 we feel justified in returning again to our original premise, namely, that none of these organisms were established *beyond question as true leprosy germs*. We therefore pass to what we have decided to designate as the period of our newer knowledge of the bacteriology of leprosy, with apologies for deciding in an arbitrary fashion where one period should leave off and another begin, or indeed whether there should exist two periods. For the sake of this presentation, however, we have found such a division most convenient.

Methods of study

It is frequently said that the progress of a science depends largely upon new ideas or hypotheses and the technical methods critically to test them. It is interesting to note that the science of bacteriology passed through its infancy and adolescence almost within the period marked by the discovery of the leprosy bacillus and the next few decades which followed. When Hansen first saw the leprosy bacillus in the tissues of lepers there were no staining methods for bacteria and only at the suggestion of Koch did he first attempt to use very dilute dyes for this purpose with most unsatisfactory but somewhat encouraging results. As new techniques were developed in the field of bacteriology they were applied to the study of leprosy as in other infectious diseases. Attempts to cultivate the germ of leprosy have been responsible for the introduction of several new bacteriological culture mediums, and without doubt the vast amount of work on new staining techniques for the leprosy bacillus has been helpful to other problems in the general field of bacteriology. As time went on various new microchemical methods were developed and applied in the study of the chemistry of suspected leprosy germs. Unfortunately, even now, the ideal methods and techniques are not available completely to settle the vexing problems which have confronted investigators in leprosy for the past sixty years. Yet there is indeed much hope on the horizon that new methods, or at least new and severe application of them, are now at hand which will eventually result in the general acceptance of recent claims which have been made for actual cultivation of *Mycobacterium leprae*.

In 1918 Wherry and Ervin (370) published a short note on the carbon dioxide requirements of *B. tuberculosis*, a problem which was later to become the subject of very extensive studies by Novy, Roehm and Soule (371) and Novy and Soule (372), not only in connection with the germ of tuberculosis, but with other organisms (see Soule (373)) as well. These investigators laid down the technical fundamentals of the study of bacterial respiration and have contributed much of what is known today regarding this interesting subject. As a matter of fact, however, the work of Hesse (374) on the gas exchange of the tubercle bacillus antedates that of Wherry by some twenty-five years since Hesse, in 1893, analyzed the air present in two cultures of *B. tuberculosis* over a period of 152 days. In more than half of his analyses the yield of CO_2 was under five per cent. Moore and Williams (375) carried on similar studies with avian tuberculosis cultures in 1909 and Corper, Gauss and Rensch (376) also determined CO_2 production in several cultures. While the studies of Wherry and Ervin did not deal with a subject entirely new by any means, nevertheless Wherry several years later (1930) employed the gaseous tension method for the first time in an attempt at the cultivation of *Mycobacterium leprae*, a study which will be referred to later on in detail. Suffice it to say at this point that the work of Novy and his colleagues, in a comprehensive study which was carried on over a period of several years, established not only the fundamentals of a critical technique but the basic laws of gaseous exchange on the part of certain bacteria, especially the tubercle bacillus. It was with the background of this work that the writer with Soule (5) (342) in 1932, described the cultivation of an organism thought by us to be the actual *Mycobacterium leprae*. This work will be described in detail later. Aside from the methods which have been mentioned in this section bacteriologists have developed no new techniques or methods of approach towards the solution of the problem of the etiology of leprosy. Let us see therefore what the past fifteen years since 1918 has produced by way of further understanding of this problem.

Cultivation of Mycobacterium leprae on solid media

In 1920 attempts were still being made to cultivate *Mycobacterium leprae*, most investigators believing that the organism had not been cultivated on artificial media with certainty. A short review of the work on cultivation was published by Zironi (377) during this year. In 1921 Kohda (378) studied Kedrowski's organism and came to the conclusion that this organism is similar to the avian tubercle bacillus, that it possesses only weak pathogenicity and that it has positive immunologic reactions against leprous serum, but it is *not* specific. The same year Richad (379) stated that the diphtheroid forms of the leprosy bacillus may be the infective form of the true organism but offered no proof of this assertion. In 1922 Walker (380) described four types of diphtheroids from leprosy tissue and concluded that the partly acid-fast diphtheroid of Bordoni-Uffreduzzi and of other authors can be cultivated from nasal and other open lesions of lepers and non-ulcerating lesions more or less constantly. Walker stated that this organism differs in size, extreme pleomorphism, peculiar colonies, fermentations and partial acid-fastness from other diphtheroids described in the literature and further that it is apparently the same as the diphtheroid cultivated from smegma and is probably a cultural form of the pleomorphic and facultative acid-fast *B. smegmatis*. The following year Walker (381) confirmed the cultivation of a chromogenic acid-fast organism from leprosy on Musgrave's and Clegg's medium and stated that neither the amoeba of Clegg nor the protein split products of Duval are necessary for growth of these organisms. He states that Clegg's acid-fast bacillus develops from Bordoni-Uffreduzzi's diphtheroid, that a chromogenic acid-fast organism like Clegg's bacillus develops in transplants from colonies of diphtheroids from smegma praeputii on Musgrave's and Clegg's medium, that a chromogenic acid-fast bacillus develops in cultures from non-leprous nasal secretions and from Hoffmann's diphtheroid isolated from such secretions on the same medium. Walker concludes that Clegg's bacillus seems to be a developmental stage of Bordoni-Uffreduzzi's diphtheroid, and that this organism seems to be identical or closely related to the pleomorphic and facultative acid-fast so-called *B. smegmatis*. In 1925 Kondo (382) studied some fourteen different strains of so-called leprosy bacilli which had been advanced by as many different investigators as the true organisms of this disease. The following year Cabral (383) published an interesting review of the entire subject but added nothing new. This was followed by another short review by Mello and Cabral (384). In 1927 de Souza Araujo (385) claimed to have cultivated the germ of leprosy on a special medium consisting of a liquid containing mannose, aluminum, ferrin and calcium and a shelf of solid medium resting above on the shoulder of a potato tube. The basic liquid medium, besides the constituents already mentioned, contained meat, water, peptone and sodium chloride. His technique was to place the leproma material on the solid media resting on the shelf above the liquid. He concluded that he had produced multiplication of *Mycobacterium leprae* and also reported the production of lesions in laboratory animals. In 1928 Kedrowski (386) published a paper in which he concluded that the leprosy bacillus, like *B. tuberculosis*, may be both acid-fast and non-acid-fast either in live tissue or on culture media; that only in exclusive cases are pure cultures of acid-fasts obtained; that in most cases diphtheroids and actinomyces-like organisms are found; and that the bacillus of leprosy should be placed in the group of actinomyces or streptothrices-like organisms. Several other authors published papers on the cultivation of *Mycobacterium leprae* during this year among them Greco (387), Jones and Tirrill (388), de Souza Araujo (389) and Keil and Unna (390). Nothing convincing is contained in any of these papers.

In 1929 Shiga (391) (392) (393) reported some experiments on the cultivation of *Mycobacterium leprae* which looked very encouraging though we understand that he is not at all satisfied (Wade) that he has finally cultivated this organism. About the same time de Souza Araujo (394) published a report in which he concluded that the leprosy bacillus belongs to the group of actinomyces. Giordano (395) then cultivated the blood of lepers on Hahn's medium and states that he obtained cultures of Hansen's bacillus which grew *vigorously* (italics ours) in subculture. In his subcultures he described ramified forms which were acid-fast and he states that this streptothrix was also present in his primary cultures.

In 1930 Wherry (396) (397) published his work in the Philippines on attempts to cultivate *Mycobacterium leprae* by the use of gaseous tensions already referred to before. This work has an important bearing on our own work to be described later and so we quote his brief note in the *Journal of Infectious Diseases* on this subject below:

> One must furnish suitable respiratory conditions as well as proper food when cultivating bacteria. Since the writer and Ervin had shown that CO_2 was essential for the growth of B. tuberculosis, and since Rockwell had demonstrated the same fact for all of a number of other bacteria, special attention was given to this fact in the attempt to cultivate Hansen's bacillus.
>
> Variations in the O_2 and CO_2 supply were brought about as follows: (*a*) Aerobic: the culture tubes were left uncapped, or when covered by a rubber cap, a fine syringe needle inserted through the cap allowed air to enter; (*b*) Little O_2 and increased amount of CO_2: the culture tubes were attached by means of gum rubber tubing to agar slants which had been inoculated with B. coli; (*c*) O and CO: The tubes were prepared as in (*b*) and then a fine syringe needle was inserted through the connecting rubber tubing and the point of the needle buried in the cotton plug of the culture tube; (*d*) No O_2 but CO_2 present: The anerobic condition was brought about by Rockwell's method (pyrogallic acid and sodium bicarbonate).
>
> The medium is prepared by boiling the white of a hen's egg in 100 cc. distilled water containing 3 to 6 percent glycerin. It is then filtered through cotton. About one-half of the yolk of the egg is boiled in the filtrate and it is again filtered, through gauze. The filtrate is autoclaved for 20 minutes at 20 pounds pressure. One cubic centimeter of this glycerinized ovomucoid yolk solution is mixed aseptically with 1 cc. of the nutrient agar to which has been added 1 to 2 drops of autoclaved oleic acid and 1 to 2 drops to autoclaved 10 percent dextrose solution in distilled water. The mixed medium is solidified in the slanting position and put in an ice box to allow water of syneresis to collect. The medium is semi-solid.
>
> I am indebted to Dr. E. V. Pineda of San Lazaro Hospital for his assistance in making the cultures. Recently discovered cases that had not been treated were chosen. The skin over the leprous lesion was cleaned with iodine and alcohol and blood containing lepra bacilli was obtained on the edge of a sterile knife by the routine "Snip" method. One loopful of blood was then transferred by means of a sterile platinum loop to the water of syneresis in the culture tubes. Control smears showed that numerous lepra bacilli were always transferred in the loopful.
>
> Variations of the above medium and a number of other media were used but in none of these did the lepra bacilli proliferate and they could no longer be recovered in smears after a few days, or after a week or two.

None of the cultures were contaminated by cocci or diphtheroids from the skin.

Proliferation is recognized only by making smears of the semi-solid culture medium. It was apparent in cultures from three cases, kept at 35° to 37°C. at the end of 4 to 6 weeks. The nuclei of planted lepra cells disappeared, and the microscopic colony-like masses of acid fasts increased in number for a few weeks and then the growth appeared to be stationary. Subculture of a loopful of material containing several dozen colonies into the same medium resulted in the appearance of a large number of subcolonies and isolated masses and scattered acid fasts. Two of the primary cultures in the above medium were successfully subcultured in the same medium, but the transplanted bacilli disappeared when they were carried over into various other modifications of the medium. In one instance, the primary culture was three and a half months old and in another instance, one month old, when the subcultures were made. The best growth was obtained in cultures which were kept first at partial oxygen tension (little O_2 but CO_2 present) for a month after which the tubes were kept under O_2 and CO_2.

The rods are thinner than tubercle bacilli and when Löffler's blue is used as a contrast strain, they often contain one or two blue granules. As in the case of smears from leprosy, if the culture preparations are first treated with xylol and alcohol the rods do not retain the stain after heating with carbol fuchsin and treating with acid alcohol. This peculiarity of lepra bacilli in smears has also been noted by E. V. Pineda.

It will be noted in Wherry's experiments that he apparently employed a very small inoculum since he used only a loopful of blood expressed from a leprosy nodule. While he states that bacilli were "numerous" in the loopful, still the inoculum must be regarded as exceedingly small as compared with most work of this nature with leprosy bacilli. Even so, and although Wherry's methods of producing his various gaseous tensions were rather crude, still he reported proliferation of *Mycobacterium leprae* in cultures from three cases, at the end of 4 to 6 weeks. He further stated that subculture of a loopful of material containing several dozen colonies resulted in the appearance of a large number of subcolonies and isolated masses and scattered acid-fasts upon his special medium. It is interesting to note that the best growth was obtained in cultures which were kept first at partial oxygen tension (little O_2 but CO_2 present) for a month, after which the tubes were kept under tension with these gases.

In 1929 Walker (398) published an interesting review on the subject of the etiology of leprosy and emphasized several new aspects of the problem. Among other conclusions Walker stated that the acid-sensitive or partly acid-fast coccoid, diphtheroid and actinomycoid organisms, that have been cultivated repeatedly from leprosy, are different stages in the life-cycle of the same organism. By 1929 the science of Bacteriology was well into the era of life-cycle hypotheses which had been gaining force since the interpretation of the phenomenon of dissociation earlier in the decade. Walker found that it harmonized more with his judgment to consider that all of these various forms of organisms which have been described in leprosy are significant etiologically to the disease of leprosy, rather than to consider them contaminants or secondary invaders as we have suggested earlier in this treatise. In this Walker may be correct though we seriously doubt it. At least it is a convenient hypothesis and one which Walker is certainly entitled to submit for consideration. The views of this author are interesting, whether right or wrong, and no doubt they have stimulated much interest in this problem. In considering the epidemiology of leprosy Walker discussed the conception of an actinomyces etiology of the disease, which had been suggested several years before, and stated that the biologic characteristics of this group of organisms suggested the possibility of the soil origin of the so-called actinomyces of leprosy. Walker undertook a bacteriologic study of the soil of Hawaii and took samples for his investigation throughout the island of Oahu. He states that acid-fast organisms were found in 98 per cent of fifty samples studied. After isolating his acid-fasts in pure culture he was able to determine that they were all of the same species and that they were extremely pleomorphic, developing cocci, diphtheroid, rod and filamentous forms and were facultative acid-fast. He further stated that these soil organisms and the organism isolated from leprosy are probably identical species of the genus *Actinomyces*. Walker concluded that leprosy is primarily an infection from the soil, probably of wounds, with this facultative parasitic actinomyces. However, he does throw in this caution, viz., that:—

> Actual proof of the identity of the actinomyces cultivable from leprosy with Hansen's bacterium in the tissues, like proof of the etiologic relation of Hansen's bacillus to leprosy, would depend upon the experimental repro-

duction of the disease in animals. Notwithstanding the absence of such proof, the evidence in support of both relations is convincing.

Walker would dispose of the great difficulty which has attended the cultivation of the germ of leprosy from the tissues of lepers on the basis that most of the organisms in the tissues are dead, a fact which may well be true, but it is not necessarily the reason why those organisms which are *living* in tissues are not easily cultivable and insufficient, if they do grow on artificial media, to start an abundant culture. We are strongly of the opinion that in these observations Walker has given bacteriologists interested in leprosy something to think about, but we also feel that there is much danger in such an hypothesis and if taken too seriously it may divert thought and action away from the more probable explanation of this disease and its true cause which we feel most strongly is not related to Walker's soil organisms. In still another paper Walker and Sweeney (399) discuss the identity of human and rat leprosy. Muir (400) takes exception to these two papers by Walker and we quote below some of his comments:

> If we examine the sources of the material from which the cultures were grown we find that the bulk of it, 442 lots out of 607, were taken in a way in which outside contamination was unavoidable—namely, from snips of unsterilized skin and scrapings from nares. Eleven of the 13 cultures of the coccoid organism were taken from a leprous nodule and a leprous bulla, and 37 of the 66 cultures of the diphtheroid were obtained from one nodule and 8 from another nodule of the same patient. In India epidermal diseases caused by actinomyces and diphtheroids are exceedingly common and presumably are also common in other warm climates. One painting of the epidermis with iodine would not be sufficient to destroy such diphtheroids and actinomyces if they were between the layers of the epidermis. Naturally, therefore, the question arises as to the technique that was used in obtaining the material. The technique used by Fraser and Fletcher when they obtained negative results in their extensive experiments was a very thorough one. They raised a flap of skin and took their material from the under surface of nodules. One wishes to know if an equally thorough technique was followed by Walker.

Muir further comments on the spread of leprosy and Walker's hypothesis as follows:

In opposition to the commonly held view that leprosy is spread by contact with infectious cases, Walker mentions, in favor of his theory that leprosy is spread by actinomyces entering the body from the soil, that there are cases of leprosy where there is no history of contact. The occurrence of such cases is, however, not to be wondered at, as cases of leprosy are not uncommon in which large numbers of Hansen's bacilli are being discharged from the nose and can be found in the skin, and yet in whom there are no outward signs of leprosy which would indicate to anyone but an expert that this disease is present. The probability is that these apparently spontaneous cases have been due to contact with such hidden lepers, contact which, when the infectee is in a state of low resistance to leprosy, does not need to be of any great duration.

In answer to Muir's criticism Walker replied as follows:

> The theory of actinomyces etiology and soil endemiology of leprosy, based on bacteriological studies and a critical examination of the endemiological evidence, was presented as alternative to the current theory of obligatory parasitism of Hansen's bacterium and the contagious spread of leprosy. Unfortunately, owing to our present inability successfully to inoculate leprosy into experimental animals, the truth of either theory cannot be proved but can only be determined circumstantially by the preponderance of evidence. We believe that the saprophytic actinomyces etiology and soil origin of leprosy better explain the known facts and obscure problems of leprosy than does the current theory of an exquisitely adapted parasite spread by contagion.
>
> Dr. Muir in his criticism has confined himself exclusively to the bacteriological evidence; it is to be regretted that he did not discuss also the endemiological evidence. He notes that a certain proportion of the pathological material was not taken aseptically, but was obtained from unsterilized skin snips and nasal scrapings. This material was collected during the routine examination of lepers, and was included in our cultural material to extend the range and as a control of the aseptically collected material. It is significant that a larger percentage of positive cultures was obtained from the aseptically collected material. Regrets are expressed by Dr. Muir that a detailed description was not given by us of the aseptic technique used in collecting the material and making the cultures. Since we did not discover any new organism, it was not considered necessary to occupy space in the description of minute details of technique. Among professional bacteriologists details of aseptic technique are a matter of routine; no aseptic technique is infallible, but we consider that ours was as adequate as is

practicable. Moreover, the parasitic origin of the organism cultivated does not rest alone on the perfection of our technique, but on the technique of scores of other competent bacteriologists throughout the world who have repeatedly cultivated the same forms from leprosy. We claim no originality for our cultures, but only for the interpretation and especially the experimental proof of the genetic relation of the different morphological and tinctorial forms cultivated from leprosy. This pleomorphic and facultative acid-fast organism is undoubtedly an *Actinomyces*, and is identical with one cultivable from the soil.

The publication of this theory of the etiology of leprosy, it was hoped, would stimulate research in this obscure disease. It is believed that the presentation of alternative and radically opposed theories of the etiology of leprosy will provide an incentive for the collection of evidence for and against the opposing theories that shall ultimately establish the truth. I am sure that in this purpose, at least, Dr. Muir and we are in entire accord.

We feel much in accord with the views expressed by Muir on this subject, particularly since Walker found it possible to cultivate his soil organisms with comparative ease while it is notorious that Hansen's bacillus cannot be easily cultivated.

About this time another idea concerning the etiology of leprosy, and one somewhat related to life-cycles of bacteria, in particular the leprosy bacillus, was introduced into the literature. For several decades those investigators who have been interested in the so-called filterable virus diseases have been the unsoliciting heirs of many diseases which have no place in this group. It finally became more or less of a habit to place any disease for which the etiologic agent could not be determined in the group of the filterable virus diseases. This has admittedly been the easiest course to pursue and the simplest way to dispose of these vexing problems. Only now are we beginning to see a little more order in the virus field and to arrive at a concept and classification of these agents. In 1932 McKinley (401) attempted to present a modern concept of the ultramicroscopic virus diseases and a classification. In this paper he devoted some attention to the question of the confusion which has existed regarding true viruses and bacteria, and pointed out that both of these groups of agents are most probably unrelated and distinctly different from each other. However, in 1929, Markianos (402) (403) (404) (405), working with rat leprosy, suggested that there is a filterable and invisible form of this organism, and pointed out that the organism first develops into primary elements and that these granules are the first visible state of the germ. Furthermore, he stated that the filterable forms of the rat leprosy organism produce the disease in rats in the same time as the bacillary forms. Markianos also stated that the filterable forms of the rat leprosy organism develop rapidly when inoculated into young rats, and that they possess an affinity for the ganglionic tissue. These forms of the rat leprosy organism will according to him pass through the pores of a Chamberland filter. Later Vaudremer, Sézary and Brun (406) reported that they had succeeded in cultivating *Mycobacterium leprae* from filterable forms of the organism. Cantacuzène and Longhin (407) also described filterable forms of *Mycobacterium leprae* and stated that the first visible forms of the organism are granules which later become typical leprosy bacilli. Similar views of the possibility of filterable forms of the leprosy bacillus playing a part in the development of the disease, or in the life cycle of the causative agent, have been suggested by others but we are hardly willing to accept these very seriously as yet, particularly in the light of our present knowledge regarding the probable nature of the true ultraviruses.

In 1930 Sonnenschein (408) published a paper on the cultivation of the leprosy bacillus on egg and glycerol and upon egg and malachite green medium. In a communication published the following year Marchoux, Markianos and Chlorine (409) stated their belief that Shiga, Wherry and they themselves had alone succeeded in sustaining growth of *Mycobacterium leprae* on artificial media. Vaudremer, Sézary and Brun (410) reported cultures of Hansen's bacillus from blood and lepromata on potato impregnated with horse serum and glycerol. These authors, however, were not quite certain of actual cultivation. Ota and Sato (411) (412) reported cultivation of Hansen's bacillus on several different media including Löwenstein's, Hahn's, Petroff's, Petragnani's, etc. These authors also cultivated *B. tuberculosis* from a leproma and a lymph gland of a leper. Pisacane (413) during the same year, reported that Hahn's medium is adaptable to the cultivation of Hansen's bacillus. At this time an interesting paper also appeared by Oliver, de Leon and Pio de Roda (414) who employed the gaseous tension method of cultivation. These authors, however, did not note any evidence of proliferation on the part of the organisms. They did note, however, the survival in one extreme instance of *Mycobacterium leprae* for 158 days. This work of Oliver and his colleagues has been commented on at some length by Soul and McKinley (415) in one of their recent publications. In 1931 Henderson (416) described a pigmented culture grown on ordinary laboratory media which he isolated from lepromata once in twenty-three attempts. No claims for etiology were made by Henderson for this organism. The following year Peschkowsky and Malilin (417) reported cultivation of Hansen's bacillus on glycerol-potato in twenty-three days. Schlossmann (418) had claimed similar results with Martin's bouillon two years before. Eichbaum (419) attempted repetition of Shiga's cultivation work with doubtful results. Denney and Eddy (420) studied the behavior of lepra and certain other acid-fast organisms in the presence of leukocytes and concluded that, of the fifty strains studied by them, only the acid-fast of rat leprosy showed evidence of globus formation. They state that acid-fast bacilli contained in "leper juice" suspended with living leukocytes did not exhibit proliferation of either free acid rods or globi but that acid-fast bacilli in pus obtained from leprous abscesses showed an increase in the number and size of the globi.

Meanwhile, to retrace our steps somewhat, there continued throughout the third decade of the century, a wide interest in the subject of leprosy. It is because this background of activity concerned with other phases of the problem did much to stimulate renewed efforts towards the solution of the etiology of the disease that we venture to call attention here to a number of the contributions occurring during those years. In 1921 Boyd and Fox (421) attempted an epidemiological study of leprosy in southern Texas; Alcazar (422) studied the history of the disease in the Holy Lands and adduced evidence to show that leprosy had existed there before the Christian era; Albert (423) reported that he believed that segregation as a method for controlling leprosy is a failure; Greenbaum and Schamberg (424) demonstrated Hansen's bacilli in material aspirated from nodules with a syringe; Serra (425) reported serological studies with the leprosy bacillus, but came to no new conclusions; Delamarre (426) brought up the old question of the absence of leprosy bacilli in nerve lesions and reported a case in which no organisms could be demonstrated; Versari (427) described a method of staining leprosy organisms with crystal violet; Rogers (428) discussed the question of incubation period in lepers and concluded that the incubation period in leprosy is between two and three years and less than formerly believed; Paldrock (429) reviewed the question of varied morphology of the leprosy bacillus. Rogers (430) also delivered the Croonian lecture on the subject of leprosy, which was an excellent review of our knowledge concerning this disease; Pineda (431) described the difference of Hansen's bacillus from other common acid-fasts; Hasseltine and Gorman (432) described the staining of Hansen's bacillus by the Schulte-Tigges method; Barbaro (433) reviewed the treatment of the disease; Riecke (434) discussed the histopathology of leprosy; Solis and Wade (435) examined some 250 children bacteriologically and found that 60 per cent were positive in the skin only and 40 per cent were positive in both skin and nose; Balbi (436) studied cholesterolemia in the three forms of leprosy; Breda (437) reviewed the question of contagion in leprosy and concluded that the disease is contagious. In 1926 Lancelin and Séguy (438) concluded that in 70 per cent of the cases of the nerve type of leprosy bacilli cannot be demonstrated. The same year Oliver (439) studied the so-called lepra cell by vital staining and found that the rat lepra cell comes from the histiocyte. He stated that he believed that the human lepra cell has the same origin; Mello and Cabral (440) examined bedbugs and flies and found leprosy bacilli in their intestines. These authors state that, after feeding on lepers, examination of the insects shows that the bacilli decrease on the fourth day and are entirely gone by the eighth day; Antunes (441) in Brazil found leprosy bacilli in 52.8 per cent of the leprous patches in 39 different lepers. He found bacilli in the nose of 9.3 per cent of 72 cases; Takeuchi (442) described bacilli in the nerve lesions of lepers; Rowe (443) in India treated lepers with vaccines of autolysed *M. tuberculosis* and reported that there was a reduction in the thickness of the nerve trunks and that anaesthetic areas lose their anaesthesia. In addition to many other excellent improvements he stated that if the treatment is continued long enough there is complete absorption of nodules.

Among the several other contemporary contributions to leprosy during this decade are papers by Paras (444) who studied the blood chemistry of lepers; Klingmüller (445) who studied the granular forms of the leprosy bacillus; Pineda (446) who reported that ten lepers out of eleven who were negative bacteriologically by nasal smear were at autopsy found to have organisms in the deeper organs. Pineda (447) also reported that of 104 placentas examined by him 57 or 53 per cent were positive for leprosy bacilli. In one case he states the organism was found in the umbilical cord and not in the placenta. Franchini (448) reported a case of *Mycobacterium leprae* septicemia. Dumont (449) described a case of tuberculosis with accumulations of acid-fasts as in leprosy.

In 1928 Vedder (450) presented a discussion of the etiology of leprosy and described his experiments in reference to the possibility of transmitting the disease by insects to two human volunteers. A third volunteer was inoculated directly with leprosy bacilli. By 1934 none of these volunteers had developed the disease. Vedder employed mosquitos in his transmission experiments and demonstrated that the insects which fed on the lepers had plenty of acid-fasts in their bodies when allowed to feed on his healthy subjects. Friedheim (451) during the following year described his work on the origin of the lepra cell and concluded that the lepra cell is a macrophage. Hoffman (452) studied the granular forms of the organism; Henderson (453) published an excellent review of leprosy; Hu and Mu (454) produced cantharides blisters on lepers and examined the fluid for leprosy bacilli. They concluded that this method yields a higher percentage of positives than the usual methods employed for bacteriologic diagnosis. In 1931 Markianos (455) prepared thick blood smears from lepers and found the organisms in thirty cases and concluded that the negative cases by blood examination are only those having the nerve type of the disease. Tisseuil (456) described tuberculoid types of the disease and states that no bacilli are to be found in these lesions. In 1932 Murdock and Hutter (457) presented a beautiful study of roentgen ray examination of lepers. Cochrane (458) published an interesting survey of the modern development of leprosy work. Lowe and Christian (459) studied various methods of diagnosis and concluded that the clip method yields the greatest number of positives. Muir and Chatterji (460) reported that the unbroken epithelium does not always prevent the escape of leprosy bacilli from the surface of the body since the organisms are found free or contained in scabs of epithelium in some cases. Cochrane (461) also published a valuable paper on the pathology of leprosy. Meanwhile statistical studies of the disease in many parts of the world were reported, such as those of Hopkins and Denny (462) in the United States.

Still another field of work that received much attention during these years was that of rat leprosy. There has for many years, been a question regarding the possible relation of rat leprosy to the human disease. In 1922 Uchida (463) concluded that rats, while susceptible to rat leprosy, are not susceptible to the human disease. He further showed that rat fleas carry many acid-fast organisms. He isolated four strains of rat leprosy bacilli, one of which produced pigment. At this same time Marchoux (464) published a paper and expressed his doubt that rat leprosy bears any relation to the human disease. Mazza (465) stated that when animals are injected with rat leprosy the organisms are taken up by the polymorphonuclear cells and distributed to the rest of the body. Sabrazès (466) also described how the macrophages in the liver take up the organisms of rat leprosy, leaving the bile free of organisms. Muir and Henderson (467) in 1928 published a comprehensive study of rat leprosy and offered further evidence that rat leprosy bears no relation to the human disease. They found that *Mycobacterium leprae* when injected into rats, Chinese hamsters and Japanese dancing mice gave completely negative results, contrary to the early reports of Duval and his coworkers with the last species. Also Muir and Henderson found that B.C.G. vaccine induced no protective effects against rat leprosy in rats. Guyon (468) reported that hereditary factors play no part in the transmission of rat leprosy and he stated his belief that the disease may be transmitted by biting. Marchoux and Chorine (469) reported that the bacillus of rat leprosy is very resistant to sulphuric acid and to sodium chloride. While this subject of rat leprosy has no particular interest in this review we quote the above papers to illustrate the range of interest in the general subject of leprosy which has been manifested during the past several years.

It will be seen from the rather hasty review which has been given above to the many articles which have appeared on various phases of the leprosy problem that the interest in the subject of leprosy has been a deep and exhaustive one in practically all parts of the world. Let us return then to the subject of cultivation of *Mycobacterium leprae* and devote some attention to the work of the writer with Soule and with Verder—investigations which we feel have given a ray of new hope, at least, to a possible solution of this problem.

We have already described the early work on the gaseous metabolism of bacteria. The fundamentals established by Novy and his colleagues on this subject have formed the basis for the approach which we have made to this problem of cultivating Hansen's bacillus. In the report of the Leonard Wood Memorial Conference on Leprosy (470) which was held in Manila in 1931, the statement appears that the entire subject of the pathogenesis of leprosy is in need of investigation. The Conference recognized clearly the necessity for the most modern research along the lines of cultivating the causative agent of leprosy, of attempting to induce the disease into animals and, among other matters, studies in the field of immunity. Stimulated by this Conference to a large extent the writer with Soule planned a detailed investigation of some of these problems and began their work in Puerto Rico early in 1931. The three reports of McKinley and Soule (5) and Soule and McKinley (342) (415) have already been mentioned. Work of the writer with Verder with tissue cultures will be described in a later section. In presenting the discussion of our own work we shall hope to be as self critical as we have taken the liberty to be with the work of other investigators, for our effort in this paper has been dispassionately to review the question of the etiology of this disease and present the facts as they seem to us to exist up to the present moment.

In 1931 Soule joined the writer at the School of Tropical Medicine in San Juan and we immediately selected a group of lepers from the Puerto Rico leper colony as a source of our material. In selecting cases we thought it advisable to choose those which had several early lesions of the disease. The cases selected were the nodular tuberculous type of the disease. The recent work of Shiga and of Wherry was before us as was also the older work of several of the most important investigators. We felt that any experimental study of this disease must necessarily take into consideration the several clinical fundamentals of the infection and that our efforts should be directed towards the problem of establishing more evidence, if possible, leading to a satisfactory fulfilment of Koch's postulates for *Mycobacterium leprae*, since all authorities seemed to agree that Hansen's bacillus is the actual cause of leprosy At this point we wish to discuss our work on the cultivation of *Mycobacterium leprae* and we will consider later in some detail our animal experimentation. I quote the following from the early paper of McKinley and Soule (5) concerning our first report on cultivation of *Mycobacterium leprae* and experimental lesions in animals:

> Leprosy has been the subject of extensive bacteriologic investigation during the six decades that have elapsed since the observations of Hansen. The bacilli noted by this worker to be constantly present in the cells of freshly excised lepromas are universally believed to be the specific cause of this malady. Notwithstanding the exhaustive attempts to cultivate *B. leprae* and the periodic reports of success, it appears to be still true that the organism has not been artificially cultivated *in vitro*. An understanding of the disease would be greatly aided by the cultivation of the leprosy bacillus, and a pure antigen would materially assist in the related serologic studies of susceptibility and the production of immunity.
>
> A review of the literature shows that a variety of organisms have been yielded on culture of leprosy material. On classification it will be seen that they fall quite naturally into three groups: (1) non-acid-fast diphtheroids; (2) chromogenic acid-fast bacilli, and (3) nonchromogenic acid-fast bacilli. What relation, if any, these organisms bear to leprosy is a question. Are they contaminating organisms that have nothing to do with the disease; or are they germs in some way associated with the true causative agent which has not as yet been cultivated (such a relationship, for example, as exists between the hog cholera bacillus and hog cholera); or are they different stages in a life cycle of the leprosy bacillus, the acid-fast phase of which is common to diseased tissue? All three possibilities have had their sponsors and suggestive experimental proof.
>
> It is of interest, however, that no organism of the aforementioned group has been cultivated with any degree of regularity from sources known to be rich in Hansen's bacillus even when the exacting requirements of the most fastidious germs were complied with. To give by way of example the results of one investigator, Walker reported 2,363 attempts in which he used over 50 different mediums, aerobic and anaerobic environments, variations in oxygen and carbon dioxide tension, in hydrogen ion concentration, in temperature, and in other conditions, with a net result that 13 coccoid, 66 diphtheroid and 1 actinomyces strains were isolated.

If, as has been suggested, Hansen's bacillus is only the tissue-invading stage in the life cycle of a pleomorphic organism, it is without precedent in bacteriologic work that such a stage, which is apparently very stable in the tissue and on the mucous membranes of the nose, cannot be isolated and maintained under artificial conditions with such attributes.

Only a few of the many investigators have considered the gaseous environment when attempting to obtain cultures of the leprosy bacillus artificially, and yet it is reasonable to believe from the recent literature on the gaseous metabolism of bacteria that consideration of the respiratory requirements may play a very important rôle in obtaining primary isolations. With this consideration in mind, the experiments to be described were undertaken.

Ten mediums, recognized as having merit for the cultivation of acid-fast organisms and used at various times in attempts to isolate *B. leprae*, were prepared as follows and placed in 18 by 150 mm. culture tubes. Witte's prewar peptone, 1 per cent, and Kahlbaum's sodium chloride 0.5 per cent, were incorporated in all the broth mediums.

Glycerol potato and glycerol broth potato. Potatoes from a number of different sources were selected and the regular slants made. A few drops of glycerol (K) were poured over the potato surface in the preparation of the former medium, and 2 cc. of 5 per cent glycerol broth were added to the slants in the latter medium, previous to sterilization.

Petroff's egg and Dorsett's egg mediums. The two mediums were prepared according to the standard methods.

Hormone glycerol agar. The broth was prepared by the infusion of fresh beef; decantation was resorted to rather than filtration at all stages in the process, and glass vessels were used throughout, 1.5 per cent agar were added previous to sterilization.

Ordinary glycerol agar. This was the usual 5 per cent glycerol, 2 per cent agar medium.

Rabbit blood agar and rabbit serum agar. Equal volumes of the melted hormone glycerol agar at 50°C. and sterile rabbits blood or rabbit's blood serum were mixed and cooled in the slanting position.

Dextrose brain broth—the usual medium of Rosenow.

Korri Konnyaku. Strips of this carbohydrate were moistened with hormone broth. The physical state of this substance was not altered by subsequent sterilization.

Patients with nodular leprosy were available. Well isolated nodules located on the arms and ears were selected, and the skin over the areas was thoroughly washed with soap and water previous to several applications of tincture of iodine. A local anesthetic was administered and four nodules from three different patients were enucleated with aseptic technic. The tissue was emulsified with physiologic solution of sodium chloride and filtered through sterile glass wool. On microscopic examination of the filtrate for the presence of acid-fast organisms, the emulsions were found rich in these forms; two drops were transferred to each tube of medium with sterile bulb pipets. Subsequently, the cotton plugs were flamed and pushed within the tubes.

When cool, the inoculated tubes were divided into four series and placed in Novy jars of 2,400 cc. capacity so that at least two inoculated tubes of each of the ten mediums, of each of the nodular series, were exposed to the various environments. The jars were closed as usual, attached to a vacuometer, and the desired gaseous atmospheres were introduced by the procedure of Novy, Roehm and Soule.

In this manner, freshly inoculated tubes were placed in atmospheres containing 10 per cent oxygen and 10 per cent carbon dioxide; 20 per cent oxygen and 10 per cent carbon dioxide; 40 per cent oxygen and 10 per cent carbon dioxide; 0.0 per cent oxygen and 10 per cent carbon dioxide, plus air controls. The early observations of Wherry and Ervin that carbon dioxide is essential to the growth of the tubercle bacillus, and the recent work of Novy and Soule, wherein it is emphasized that a definite concentration of free carbon dioxide is absolutely necessary to maintain the physicochemical equilibrium between the extracellular and the intracellular carbon dioxide, directed the use of free carbon dioxide in these attempted isolation experiments of the leprosy bacillus. From previous work with bacteria and protozoa, a concentration of 10 per cent carbon dioxide seemed to be the most favorable for the primary isolation of organisms; therefore this tension was introduced in all the jars in the present series of experiments, and the oxygen concentration was varied.

The carbon dioxide was prepared in a Kipp generator from the interaction of marble and dilute hydrochloric acid. The gas was washed previous to its introduction into the jars by passage through a saturated solution of sodium carbonate. The oxygen was obtained in the small tanks as used for medical purposes. Hydrogen was made in a Kipp generator out of purified zinc and dilute hydrochloric acid, and washed by passage through solutions of silver nitrate, sodium carbonate, lead acetate and alkaline pyrogallate.

To obtain the desired tensions of the different gases, the jars were attached to the vacuometer and after exhaustion to the desired negative pressures the calculated number of millimeters of the gases were run in. No buret analyses were made, but previous experience with this method of producing gaseous mixtures of definite composition indicated that the tensions desired were obtained with a variation of less than 0.5 per cent.

After the adjustment of the gaseous tensions, the jars were placed in incubators at 37.5°C. The ordinary air controls subsequent to the flaming of the tubes were sealed with wax, and a tiny hole was put through the wax with a hot wire to insure an interchange of air, as suggested by Novy and Soule in their studies on the respiration of the tubercle bacillus. These tubes were then incubated at 37.5°C. in close proximity to the jars.

The air controls were carefully observed for evidence of growth and contamination. The jars were regularly removed from the incubators and inspected, but they were not opened during these routine examinations. At the end of four weeks of incubation the jars were opened, and several contaminated tubes containing a spore-forming aerobe were discarded. There was no macroscopic evidence of growth in the uncontaminated tubes. Several were therefore selected, and smears were prepared from material obtained from the surface of the medium and stained by the Ziehl-Neelsen method. Microscopic examination indicated proliferation in several tubes; therefore the tubes were returned to the jars, the jars closed and the original gaseous mixtures introduced and incubation continued. At the end of six weeks of incubation the jars were opened and all tubes were carefully examined microscopically and macroscopically. The data are presented in table 1.

The tubes were taken from the jars and the surfaces of the mediums were carefully examined by transmitted and reflected light for colonies. When present, the colonies were small, averaging about 1 mm. in diameter, and heaped up, with a distinct mucoid appearance and a loose filamentous border. Whether or not colonies were present, at least two smears were made from material taken from the surface of the medium and from the water of condensation in each tube. The preparations were air dried gently fixed and stained with carbolfuchsin, decolorized with 10 per cent sulphuric acid and counterstained with Loefflers' methylene blue (methylthionine chloride U. S. P.). Tubes which showed the presence of colonies and well formed, solid-staining rods were designated as plus; there was a total of forty-six tubes in this group. Frequently, in the absence of colonies, the smears contained well formed solid-staining rods suggestive of proliferation or at least of a favorable reaction to the new environment; such tubes were noted as containing a questionable growth. When the preparations contained only granular acid-fast bodies or highly granular rods, zero growth was recorded. No bacillus, coccus or actinomyces types were observed in any of these tubes or in the subsequent cultures, excepting the aerobic sporeforming contamination noted in the ordinary glycerol medium.

A comparison of the data presented in table 1 should be made. It will be noted that no medium or gaseous environment gave uniformly positive results. However, there seemed to be a distinct advantage in the use of the glycerol potato, the egg-containing and the hormone glycerol agar mediums. The most favorable gas environment seemed to be 40 per cent oxygen and 10 per cent carbon dioxide, although in the presence of 10 per cent and 20 per cent oxygen plus 10 per cent carbon dioxide there were many positive results. It was rather to be expected from the experiments of Wherry that the low oxygen and 10 per cent carbon dioxide environment would give the best results, but such was not the case. The observed fact that 40 per cent oxygen and 10 per cent carbon dioxide was the most favorable environment is in accord with previous work on the tubercle bacillus, wherein it was found that this increased tension of oxygen was the most favorable for the growth of this germ.

TABLE 1

*Growth on mediums used for isolation of B. leprae in various concentrations of oxygen and carbon dioxide in Novy jars incubated at 37°C. for six weeks**

	PERCENTAGE OF CO_2 AND O_2																AIR CONTROLS			
	O_2 10.0– CO_2 10.0				O_2 20.96– CO_2 10.0				O_2 40.0– CO_2 10.0				O_2 0.0– CO_2 10.0							
Patient	1	1A	2	3	1	1A	2	3	1	1A	2	3	1	1A	2	3	1	1A	2	3
Mediums:																				
Glycerol potato	?	?	0	0	+	?	0	+	+	+	0	+	0	0	0	0	0	0	0	0
Glycerol broth potato	?	0	+	0	+	?	?	0	+	+	0	?	0	0	0	0	0	0	0	0
Petroff's egg	?	+	+	0	+	+	?	+	+	+	+	+	0	0	0	0	0	0	0	0
Dorset's egg	0	0	0	0	0	0	0	0	+	+	?	0	0	0	0	0	0	0	0	0
Hormone glycerol agar	+	?	0	+	+	+	?	+	+	+	+	?	0	0	0	0	0	0	0	0
Ordinary glycerol agar	0	0	†	0	0	†	†	0	0	†	0	†	0	0	0	0	0	0	0	0
Rabbit blood agar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit serum agar	0	?	0	0	0	?	0	0	+	+	0	0	0	0	0	0	0	0	0	0
Dextrose brain broth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Koori Konnyaku	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Those tubes showing positive growth of acid-fast rods were subcultured to same mediums and returned to previous gaseous environments; + indicates tiny colonies with well formed rods; ? indicates well formed rods with question of colonies; 0 indicates highly granular degenerating rods.

† Contamination.

A striking fact is that no growth took place in any of the air controls or in the tubes incubated under anaerobic conditions plus carbon dioxide. The air controls should be compared with the tubes incubated in air plus 10 per cent carbon dioxide, which mixture seemed to be second best for the isolations. Thus, one is to infer that the presence of free carbon dioxide is beneficial for the obtaining of primary isolations. From the absence of growth in the oxygen-free jars (and it might be mentioned at this point that the granulation of the cells was most conspicuous in those tubes in the anaerobic jars), it may be inferred that the leprosy bacillus is a strict aerobe. It is possible, and, indeed, later experiments have demonstrated, that the germ can utilize free oxygen at concentrations as low as 1 per cent, if carbon dioxide is present and, of course, provided a sufficient amount of oxygen is available at this low tension.

The growth from sixteen of the tubes marked plus was taken up in physiologic solution of sodium chloride, and this suspension, rich in acid-fast organisms, was used for the inoculation of the monkeys.

Fresh potato, egg and hormone glycerol agar mediums were prepared, and the growth from the remaining thirty positive tubes was transferred with a platinum wire to the freshly prepared mediums; the growth on each medium was transferred to a like tube of medium. After the cotton plugs were flamed and cooled, the tubes were returned to the jars and the gaseous mixtures which had favored the initial growths were introduced. The jars were then placed in the incubator at 37.5°C. for four weeks. At the end of the incubation period the jars were opened and the tubes were examined macroscopically and microscopically for growth as before. Only eleven tubes in this second generation gave the typical colonies observed in the primary isolations and again the 40 per cent oxygen and 10 per cent carbon dioxide mixture seemed to be the most favorable atmosphere; the positive cultures were about equally distributed among the three varieties of mediums.

The growth was removed from the eleven positive tubes and placed on the surface of freshly prepared tubes of medium and returned to the jars with their former gaseous mixture, and incubation was again carried out for four weeks. It was noted on the examination of these tubes at the end of the incubation period that colonies were present in ten of the tubes, but these so-called third generation colonies were no larger than the colonies obtained in the primary isolations; in other words, the germs were not rapidly adapting themselves to a saprophytic existence.

Subcultures were made from the ten tubes to like mediums and the freshly inoculated tubes were returned to the same gaseous environments, incubated as usual and examined at the end of four weeks. When the jars were opened, only five of the tubes showed the typical colonies. These positive tubes were subcultured to about twenty tubes of each of the three favorable mediums, and the freshly inoculated tubes were placed in the jars and 40 per cent oxygen and 10 per cent carbon dioxide were introduced, and incubation was carried out as before. This is the status of the cultivation experiments to date.

A recapitulation of these experiments shows that there were forty-six positive cultures in the first generation, eleven in the second, ten in the third and five in the fourth. These data are suggestive of a gradual loss in the power of adaptation of this acid-fast organism to growth on artificial mediums.

We believe that these experiments confirm the recent work of Shiga and also of Wherry on the isolation of an acid-fast organism from human leprosy, with perhaps much more satisfactory cultural data.

In a more detailed communication Soule and McKinley (415) were able to present a later report on these cultural data after the organisms had been carried through the sixteen generations of subcultures. In this report we listed the number of positive cultures in each generation subculture from the first to the sixteenth as follows:

46 positive tubes in generation 1
11 positive tubes in generation 2
10 positive tubes in generation 3
5 positive tubes in generation 4
8 positive tubes in generation 5
6 positive tubes in generation 6
6 positive tubes in generation 7
7 positive tubes in generation 8
5 positive tubes in generation 9
5 positive tubes in generation 10
3 positive tubes in generation 11
3 positive tubes in generation 12
6 positive tubes in generation 13
5 positive tubes in generation 14
3 positive tubes in generation 15
2 positive tubes in generation 16

It is apparent from the number of positive cultures in these sixteen generations that the organism cultivated by us from leprosy patients was gradually losing its ability to multiply under the artificial conditions imposed upon it, though it was still living after sixteen generations and forming definite colonies. By the latter part of 1933 the organism had been carried through ten additional subculture generations making a total of 26 generations in all and cultivation over a period of over two years and a half.

In this second report we also reported our experience with other media including those advocated by Twort, Petragnani, Wherry and vegetable and amino-acid mediums. Growth did not occur on any of these media except in two tubes of Twort's medium. We also described our attempts at serological studies with our culture of *Myco-*

bacterium leprae but obtained no evidence of specificity with the precipitin test. Complement fixation was positive in nine of twelve sera tested and agglutination was positive in dilutions of 1:20 to 1:50 in only four out of twelve leper sera. Six control sera were entirely negative however. Further data on animal experimentation were also described and these will be mentioned later in a section dealing with this subject. The least which can be said of this early work of Soule and the writer is that it was encouraging.

Cultivation of Mycobacterium leprae in tissue culture

Early in 1933 McKinley and Verder (471) described a method of cultivating *Mycobacterium leprae* in minced chick and also human embryonic tissue suspended in Tyrode's solution We found that multiplication of *Mycobacterium leprae* takes place in such medium under ordinary atmospheric conditions but it is probable from our more recent experience that these cultures also do much better when they are carried under O_2 and CO_2 tension, for the pH of the medium can be much better controlled in this manner. Later during this year McKinley and Verder (472) described the cultivation on solid media, under gaseous tension, of *Mycobacterium leprae* from tissue culture. The appearance of minute micro-colonies was interpreted as direct evidence of the multiplication of the organisms in tissue culture. We have had considerable experience in attempting to enrich our tissue culture medium but so far we have found no method which will ensure a much better growth than we have reported. It is interesting to note that in the Annual Report of the Surgeon General (473) of the United States Public Health Service for 1932 there is a report under "Leprosy" of the use of chick embryo tissue culture for the cultivation of *Mycobacterium leprae.* This report states that "in three instances of the cultures of human material there has apparently been a proliferation of the acid-fast bacilli planted and a definite growth of a diphtheroid in from five to seven days after inoculation." The report further states that all of these cultures were carried through several transplants, and one of them through 15 transplants, and the acid-fasts in the last transplants seemed to be as numerous as in the original culture. While this report was not available to Verder and myself when we first published our work on tissue culture it is of great interest to us in retrospect since our first paper was in press when, presumably, the report quoted was in manuscript. The results of the workers of the Health Service coincide in some respects at least with our own investigations. The work on tissue culture is being continued at the present time in the hope that soon the ideal method of cultivating *Mycobacterium leprae* may emerge from such studies.

More recently Soule (474) has returned from the Culion leper colony where he carried on investigations under the auspices of the Leonard Wood Memorial for the Eradication of Leprosy and he reports, in a personal communication, the following regarding his work on cultivation of *Mycobacterium leprae*:

> The cultural studies are arranged under four headings: (1) the inoculation of normal mediums with "Lepra Reaction Pus" rich in acid-fast forms, fluid aspirated from broken down nodules and freshly ground up nodules with subsequent incubation in an atmosphere enriched with 10 per cent CO_2 and 40 per cent O.
>
> (2) Repeating the experiments of McKinley and Verder with the additional precaution of using autoclaved suspensions as controls to avoid the objection of confusing proliferation with mechanical transfer.
>
> (3) The preparation of chick embryo plasma cultures, using guinea pigs plasma according to method of Carrel in Carrel flasks, again controlling with autoclaved material.
>
> (4) Attempts to cultivate infected human tissue according to the methods of Lewis.
>
> Results: An attempt was made to cultivate organisms from a total of 54 specimens of infected material. With 4 exceptions (tuberculoid) direct stains of the samples of the original material showed an abundance of many solid stained acid-fast rods.
>
> By the first method, positive growth appeared in several tubes in twelve out of twenty specimens. The growth was typical as described by Soule and McKinley—tiny, flat, transparent, pin-head colonies which never became large but were viable through three generations.
>
> Twenty-six attempts were made using the technique of McKinley and Verder. There were four failures to obtain positive growths. The preparations were subcultured through six generations.
>
> Only eight attempts were made to culture the material by the third method. In every instance there was unquestioned reproduction but time did not permit the carrying through of this method to any great extent.

The fourth method was utilized in only two instances and was not given a fair trial.

As regards some general considerations: 1. No colonies or growth appeared at any point in this study other than that described by Soule-McKinley, McKinley-Verder. The organisms were distinctly acid-fast. A careful check was made for the presence of diphtheroids and rapidly growing chromogenic acid-fast forms with negative results.

It would seem, from the review of the work of Soule with the author and Verder and the author, and the recent independent work of Soule in the Philippines, that there is a mass of rather encouraging data being assembled concerning experimental work on the problem of cultivation of *Mycobacterium leprae*. However we trust that we may continue and be permitted to maintain the conservative point of view which we have so far tried to express in our various publications regarding this question. We are under no illusions regarding this question which has been one of perennial controversy almost since Hansen first saw the leprosy bacillus in the tissues of the leper. We realize that Koch's postulates have not been entirely fulfilled as yet. However, we feel that in the organism isolated by Soule and the writer we have something quite distinct from the ordinary organisms which have been described as *Mycobacterium leprae*. This organism is one of an extremely delicate constitution apparently. It is most difficult to cultivate and so far we have found only two methods which will succeed in so far as artificial cultivation is concerned. Perhaps this difficulty in isolating it, and the difficulty of maintaining it under cultivation after it is isolated, are the two main points in its favor. At least these characteristics seem to harmonize fairly favorably with the history of the organism. The fact that this organism is also a non-chromogenic bacillus is also in its favor as is also the fact, as we shall see later, that suggestive lesions may be produced with it in experimental animals. The author and his colleagues, however, feel that their reports should be tested thoroughly and rigidly by other investigators.

Animal experiments and results

To return to 1919 again, we find that during the past fifteen years many reports have appeared in the literature regarding the production of suspicious lesions of leprosy in laboratory animals. In 1919 Bradley (475) described the production of rather extensive nodular lesions of leprosy in the *M. rhesus* monkey. Marchoux (476) described an acid-fast bacillus infection which he believed to be neither tuberculosis nor leprosy. Valvêrde (477) published a short review of the literature on transmissibility of this disease. Maucione (478) reported further experiments on the inoculation of rabbits with leprosy material in the anterior chamber of the eye and produced definite opacities in the cornea. Limousin (479) performed similar experiments with rabbits and repeated his inoculations six months later and kept his animals 22 months before sacrificing them. At autopsy he found no lesions except in the lungs where there were nodules rich in acid-fast bacilli which he believed to be leprosy organisms. Reenstierna (480) reported further experiments claiming the production of leprosy lesions in monkeys which developed 39 days following inoculation. Banciu (481) (482) inoculated rabbits intravenously with leprosy tissue emulsion but apparently produced no lesions in these animals following such a procedure. He also studied the sera for fixation but could demonstrate none. In other rabbits he injected the sera of lepers intravenously and the rabbit sera retained fixation properties for a few hours but these were entirely gone in 24 hours. In a dog fixation properties of the serum following such injections remained for as long as 48 hours. Mariani (483) injected virulent and killed leprosy material intradermally into man but produced only various grades of reactions without lesions. This was in 1925. In 1926 Reenstierna (484) again reported experimental lesions in monkeys. He employed both *M. rhesus* and *M. sinicus* monkeys (see illustrations). The following year Roffo (485) attempted the transmission of leprosy to African monkeys and American Cebus monkeys and produced most interesting lesions (see illustrations). The same year Muir, Henderson and Landeman (486) reported a beautiful study of rat leprosy in rats and pointed out that this organism is only related to the human species as avian and bovine tuberculosis are related to human tuberculosis. Franchini and Cendali (487) studied the possibilities of producing lesions of leprosy in the white rat but without convincing results. In 1928 de Souza-Araujo (488) (489) reported local cutaneous nodules in the white mouse which he believed typical of human leprosy though later this author did not feel so convinced that he had actually produced the experimental disease in this animal. Further experiments with monkeys were reported by Franchini (490) in 1929. In this work this author described nodular lesions in the *M. sinicus.* In 1930 Tisseuil (491) injected man with both *Mycobacterium leprae* and *B. puliforme* intrademally and he reports that slowly developing abscesses were produced. The latter organism alone also produced abscess, but *Mycobacterium leprae* alone did not produce the lesion. The same year another report of Franchini (492) described the last stages of experimental leprosy in a monkey which had been inoculated more than three years previously. He states that the animal died in a state of general decline and was suffering from paralysis of its hind legs. A few months previous to death a leprous nodule had developed in the area of original inoculation and many Hansen bacilli were found in this lesion. About the same time Schöbl, Pineda and Miyao (493) in the Philippines reported the results of their experiments with experimental leprosy in the Philippine monkey. These authors gave subcutaneous injections of leprous material over the eyebrow and repeated the inoculations in the same locality. They concluded that there is an allergic factor involved which assists in the production of lesions. In 1931 Borrel and Larrousse (494) infected rats with both leprosy and the cysticercus of *T. crassicola* and reported that there was brought about a localization of leprosy in the livers of these animals. Further papers on the experimental transmission of leprosy to animals were published recently by de Souza-Araujo (495), Cantacuzène and Longhin (496) who claimed to have produced lesions in the white rat and Ota and Sato (497) (498) who described lesions in white rats four to five months following inoculation. In 1932 Pinoy and Fabiani (499) described their failure to produce lesions of leprosy following intraperitoneal injection of leprosy material in a splenectomized monkey. Finally in the report of the Surgeon General of the United States Public Health Service (500) for 1931 there are described experiments attempting to infect rats with both human and rat leprosy material by dropping infective material into the nose. It is reported that both of these organisms apparently penetrate the nasal mucosa, for acid-fast organisms are later to be found in the cervical lymph nodes, in the lungs and in the spleens of animals so treated. This report also states that granulomatous tissue changes were produced in kittens following injection with material from lesions of both human and rat leprosy.

Reference has already been made to the recent experiments of the writer and Soule (5) (342) (415) and we quote below one of these reports in some detail dealing with our experimental work with animals.

> In the leper colony of the Insular Department of Health of Porto Rico there are sixty patients with leprosy, both male and female, and it is estimated that there exist perhaps no more than forty other lepers on the island who are not under treatment. From the male patients at the leprosarium we selected a group of five showing marked nodular lesions. All experiments which we had carried on previous to those reported in this paper, which were designed to induce lesions of the disease in monkeys and other laboratory animals, had failed. Monkeys, rabbits and guinea-pigs have, in the past, been inoculated by us with emulsified leprosy nodules by several methods (intratesticularly, intraperitoneally, intramuscularly, intracerebrally and through other routes) and with one exception, the animals have remained in good health and have showed no evidences of the disease. One monkey became ill and died a year following intratesticular inoculation with leprosy material and on autopsy was found to have succumbed to generalized tuberculosis. The work of recent investigators, however, indicates that experimental lesions may be produced in monkeys following intradermal inoculation of leprosy material. We therefore decided to utilize the intradermal route of infection and by this method we succeeded in producing granulomatous lesions very suggestive of leprosy in both Macacus rhesus and Cebus olivaceus monkeys.
>
> The lepers were brought to the University Hospital of the School of Tropical Medicine, and typical nodules from the arms and ears were removed under rigid aseptic technic by Dr. William R. Torgerson of the surgical staff. These nodules were dissected carefully from the underlying tissues and removed without skin. In the laboratory they were ground up finely with glass rods in sterile test glasses and emulsified in physiologic solution of sodium chloride. The various emulsions were examined by stained preparations for acid-fast organisms and were found to be rich in acid-fast bacilli, presumably *B. leprae* Hansen. Cultures were then prepared on mediums which will be described later. The emulsions were then pooled and eight *Macacus rhesus* and five *Cebus olivaceus* monkeys were

inoculated intradermally over the eyebrow with 0.25 cc. of the material (table 2).

It will be noted in table 2 that in seven of the eight *Macacus rhesus* and in all five of the Cebus monkeys, nodular lesions developed at the site of inoculation in from eighteen to twenty days following injection of the leprosy material. These nodules were firm, hard and red, and did not show any tendency to soften. However, within a week following the appearance of the nodules, one of the nodules ulcerated and another was doubtful, because it was thought that the animal might have injured himself at the site of inoculation in moving about the cage. From the latter, some serum was expressed from the lesion and acid-fast bacilli were demonstrated in the stained smear. The ulcerated nodule was removed for histologic study, as were several of the other most typical nodules. Parts of the nodules were emulsified, and cultures and stained smears were made with this material. The smears showed numerous acid-fast bacilli. The appearance of such lesions in monkeys following the inoculation with leprosy material from human beings is in accordance with the work reported by several other investigators who have reported incubation periods ranging from eighteen to seventy days. The shortest incubation period in monkeys which correspond with the experiments described in this paper has been reported by

TABLE 2

*Inoculation of monkeys with human leprosy material**

MONKEY	RESULT
M-1	Nodule developed in 18 days
M-2	Negative
M-3	Nodule developed in 19 days
M-4	Nodule developed in 18 days
M-5	Nodule developed in 18 days
M-6	Nodule developed in 20 days
M-7	Nodule developed in 18 days
M-8	Nodule developed in 18 days
C-1	Nodule developed in 19 days
C-2	Nodule developed in 20 days
C-3	Nodule developed in 20 days
C-4	Nodule developed in 19 days
C-5	Nodule developed in 18 days

* In this group each animal was inoculated intradermally with 0.25 cc. of human leprosy nodule emulsion.

Kryle, while the longest periods are those reported by Nicolle (sixty-two days) and by Bradley who reports an incubation period of ten weeks.

Histologic examination by Dr. William C. von Glahn of sections of three of the most typical nodules revealed definite granulomas consisting of nodular accumulations of cells of the large mononuclear type with infiltrations of lymphocytes and clumps of polymorphonuclears. The characteristic foamy cellular cytoplasm was not present in these early lesions and the first acid-fast stains of these sections revealed no bacilli, although subsequent study of these sections with a modified technic showed the acid-fast organisms and acid-fast granules to be present. Multinucleated giant cells were found in some of the sections. The lesions were distinctly early and the histologic pictures presented are suggestive of early lesions of leprosy which, we believe, they may be. However, such lesions in monkeys, in our experience, vary greatly in size, ranging from 0.5 to 2 cm. in diameter, and increasing gradually during the first ten days or two weeks following their appearance. They then tend to regress, and they disappear entirely within another three or four weeks. Schöbl, Pineda and Miyao reported the production of nodular lesions in the Philippine monkey by repeated intradermal inoculation of human leprosy material and they believe that there is an allergic factor involved in the production of such lesions. Our experiments would not indicate this, since, in the majority of monkeys of the two species we have used nodular lesions are produced following a single injection of the leprosy material. This also coincides with the experiments reported by Reenstierna who has described experimental lesions in monkeys practically identical with our own.

In view of the several reports of other investigators concerning the experimental production of granulomatous lesions in monkeys with human leprosy material and the positive results we have obtained in our experiments, it seems most likely that in these lesions we have definite evidence of experimental transmission of the infection of this experimental animal. The monkey, however, apparently possesses considerable natural immunity to this infection, for such experimental lesions are not progressive and usually the infection is aborted within a few weeks following the appearance of nodular lesions. Control animals have been inoculated with mediums and various other substances, such as wax and ground guinea-pig lymph gland, and no lesions have apparently resulted from such inoculations.

One of the most typical nodules produced in this series of a *Macacus rhesus* was removed for histologic study, and part of the nodule was emulsified for the purpose of attempting to pass the infection in series in other monkeys. The emulsion contained acid-fast bacilli but they were not numerous. Three normal *Macacus rhesus* monkeys were inoculated intradermally over the eyebrow with 0.25 cc. of this emulsion, but in none of these animals did lesions develop. We are at a loss to account for the re-

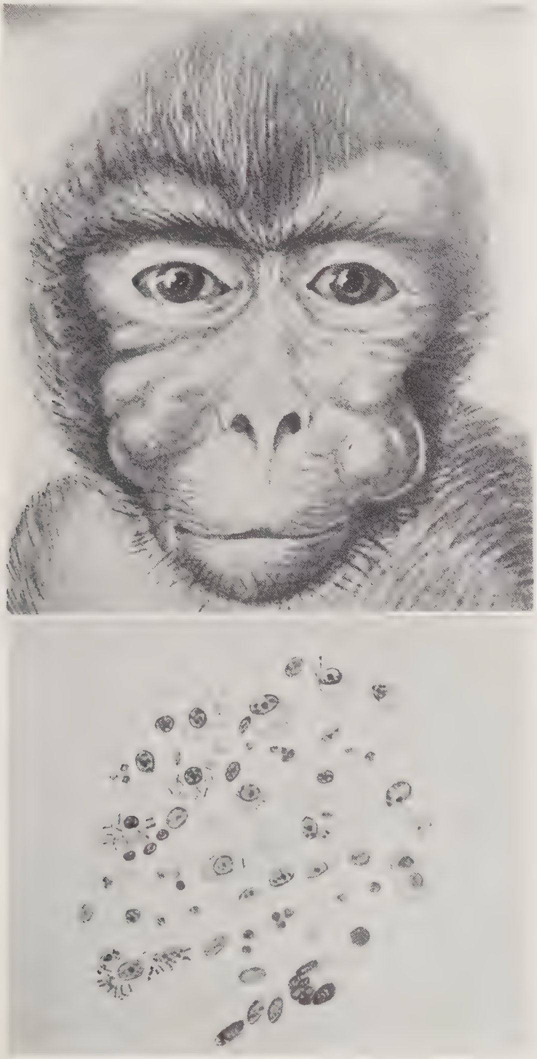

FIG. 1. TYPICAL NODULAR LESIONS PRODUCED IN A MONKEY WITH LEPROUS MATERIAL AND SMEAR FROM LESION SHOWING TYPE OF CELLS AND PRESENCE OF ORGANISMS

(From Reenstierna)

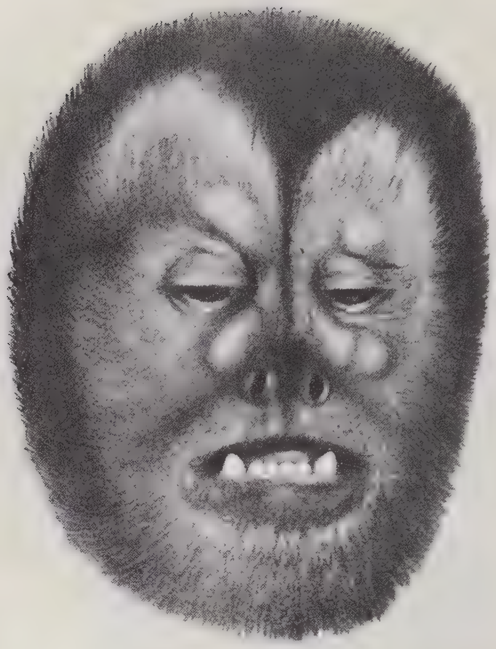

Fig. 2. Typical Nodular Lesions Produced in a Monkey with Leprous Material
(From Roffo)

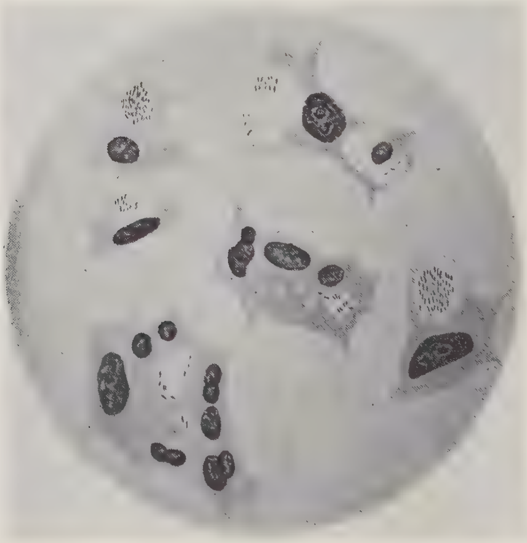

Fig. 3. Types of Cells and Accumulations of Organisms Found by Reenstierna in Experimental Nodular Lesions
(From Roffo)

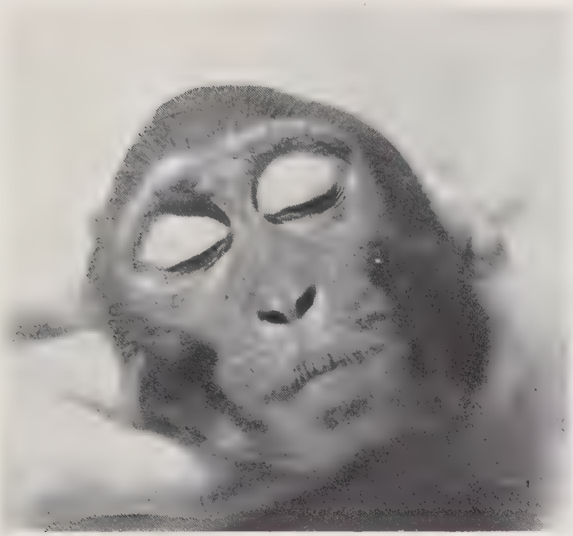

FIG. 4. TYPICAL NODULE ON MACACUS RHESUS FOLLOWING INOCULATION WITH HUMAN LEPROSY MATERIAL. NODULE DEVELOPED IN EIGHTEEN DAYS

(From McKinley and Soule)

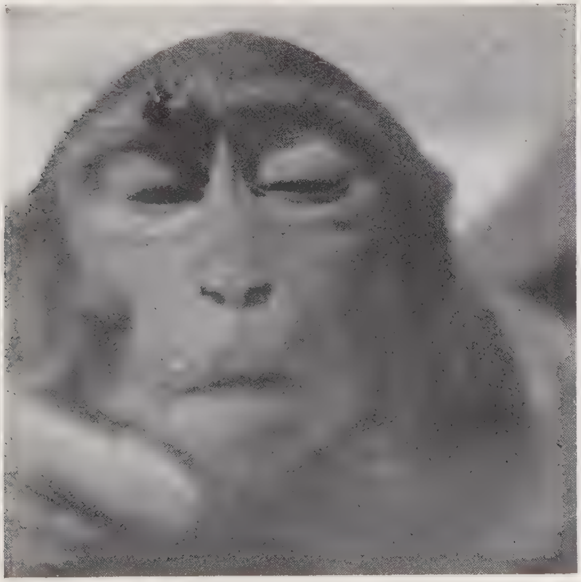

FIG. 5. SAME MONKEY AS IN FIGURE 4, SHOWING ULCERATION OF NODULE ELEVEN DAYS AFTER APPEARANCE OF NODULE

(From McKinley and Soule)

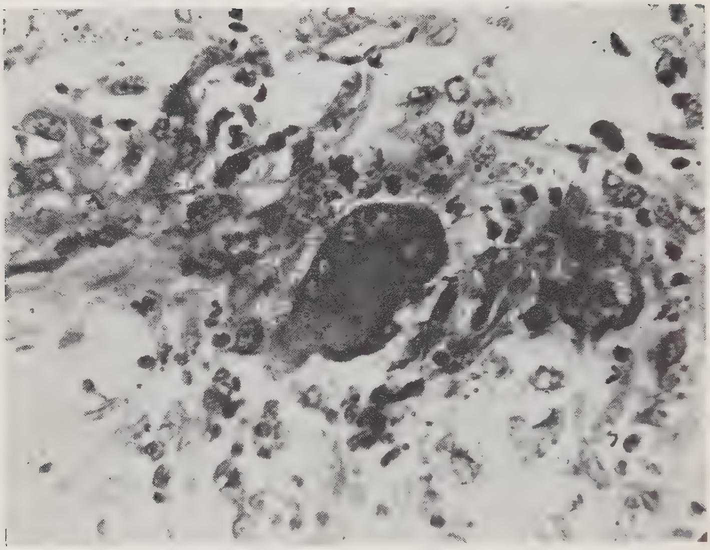

Fig. 6. Section of Nodule Showing Granulomatous Lesion
(From McKinley and Soule)

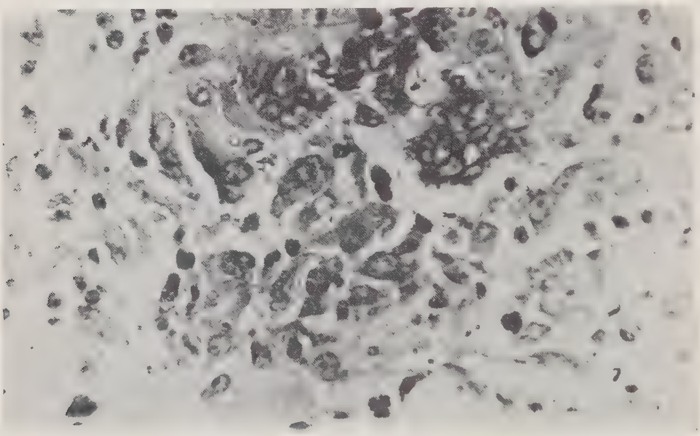

Fig. 7. Section of Nodule Showing Granulomatous Lesion
(From McKinley and Soule)

sistance manifested by these animals, but we should point out that the emulsion did not contain the massive infection which was given to the first series of animals in direct inoculation from man, and it is apparent that the new host was able to resist the implantation of the amount of infection that was given. These three animals do, however, illustrate that the mere injection of ground nodular tissue (even when containing some acid-fast bacilli) is not followed by the appearance of the typical nodules which we have described as a result of inoculation with human leprosy material. They may therefore be regarded as additional controls.

Experimental lesions in monkeys with cultures of B. leprae

With definite proliferation of acid-fast bacilli in cultures prepared with the leprosy nodule emulsions it remained to determine whether nodular lesions could be produced experimentally in monkeys with the artificial cultures of the organisms. Ten *Macacus rhesus* and seven *Cebus olivaceus* monkeys were inoculated intradermally over the eyebrow with 0.5 cc. of a pooled suspension of acid-fast bacilli taken from several cultures. Guinea pigs were also inoculated intraperitoneally with 1 cc. of this suspension to rule out Bacillus tuberculosis. Results of inoculations with cultures of acid-fast bacilli from leprosy nodules grown on artificial mediums are found in table 3.

In five of the *Macacus rhesus* and five of the *Cebus olivaceus* monkeys which were inoculated intradermally with cultures of acid-fast bacilli, nodules developed in from one to two weeks following inoculation, varying in size from that of a small pea to 1 cm. in diameter. The nodular lesions were firm, hard and somewhat reddish, and tended to regress rapidly after the third or fourth week. There was no evidence of secondary infection or suppuration. From Cebus 1, smears were prepared from the nodule over which the skin was broken by injury in handling the animal and these smears showed numerous acid-fast bacilli with some mononuclear and polymorphonuclear cells. Sections of the nodules excised for histologic study showed definite granulomatous changes.

One of these nodules, which was seen by Dr. Von Glahn, showed a marked cellular infiltration consisting of large mononuclears, polymorphonuclear cells and lymphocytes; marked edema and occasional multinucleated giant cells were noted. The central cytoplasm of some of the giant cells was stippled but not definitely foamy in character. Acid-fast organisms could not be demonstrated in these sections. In other sections of nodules produced by inoculating cultures, we have seen occasional acid-fast bacilli but they have been remarkable for their scarcity, though some of the sections removed on the thirty-first day have shown cells which were definitely becoming slightly foamy in character. These lesions, however, as the lesions produced experimentally in monkeys with direct inoculation of leprosy material from lepers, described in the beginning of this report, are very early lesions. Our experience with such lesions, however, is limited and no studies are available in the literature on the histology of such early lesions. We feel, therefore, that both types of lesions should be classified as granulomas which are very suggestive of early lesions in leprosy.

TABLE 3

*Inoculation of monkeys with cultures of B. leprae**

MONKEY	RESULT
M-1	Small nodule (21 days)
M-2	Negative
M-3	Negative
M-4	Small nodule (7 days); much larger (14 days). Excised
M-5	Negative
M-6	Small nodule (7 days)
M-7	Negative
M-8	Negative
M-9	Small nodule (7 days); much larger (14 days). Excised
M-10	Small nodule (7 days); much larger (14 days)
C-1	Small nodule (7 days)
C-2	Small nodule (7 days); much larger (14 days)*
C-3	Small nodule (14 days)
C-4	Negative
C-5	Small nodule (7 days); much larger (14 days)
C-6	Small nodule (7 days)
C-7	Negative

* In this group each animal was inoculated intradermally with 0.5 cc. of culture suspension of *B. leprae.*

As a result of these studies Soule and the author commented as follows:

> The reports of other investigators on the experimental production of nodular lesions of the granulomatous type in monkeys following inoculation with human leprosy material seems to be well founded. Our experiments would indicate that one may with a great deal of regularity infect certain monkeys with such material by intradermal inoculation and produce lesions within three weeks at the site of injection which are suggestive of lesions of early leprosy. Acid-fast bacilli are to be found in such lesions as

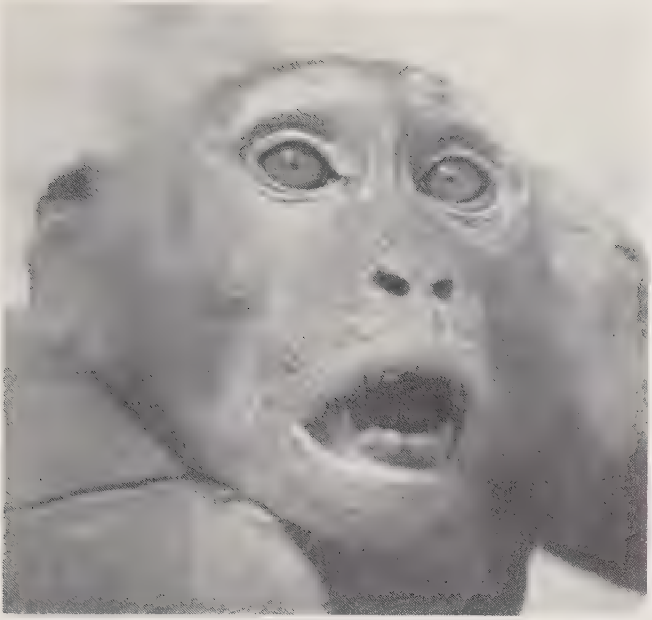

Fig. 8. Typical Nodule in Skin of Macacus Rhesus Monkey, Produced with Culture of Acid-fast Organisms from Human Leprosy Material
(From McKinley and Soule)

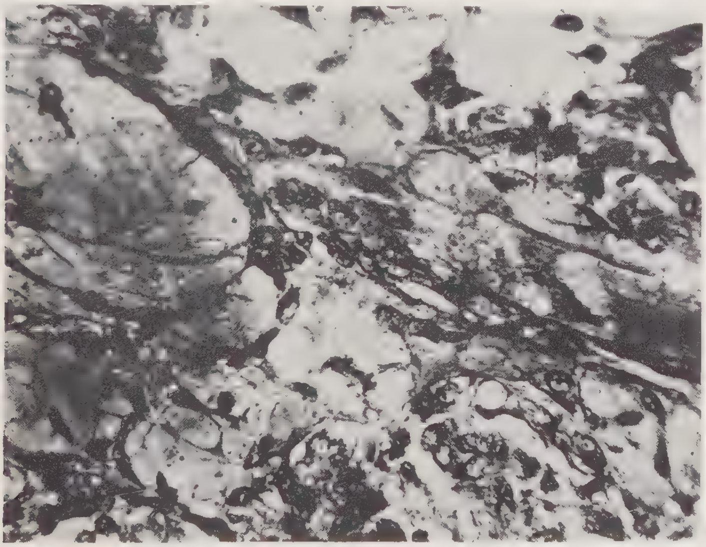

Fig. 9. Section of Nodule Removed from Monkey in Figure 8, Showing Granulomatous Lesion and Beginning Foam Cell Formation
(From McKinley and Soule)

other workers have observed. The entire question of the cultivation of *B. leprae* should be studied further, with utilization of recent methods which have been advocated. Our experiments have led, we believe, to the unquestionable cultivation of *B. leprae* on artificial mediums. Experimental lesions have been reported by several investigators in such animals as guinea-pigs, rats, the Japanese dancing mouse, and other mice. None of these lesions have been definite enough to permit their acceptance as evidence of experimental leprosy in these animals. Clegg was unable to infect monkeys with his cultures, although guinea-pigs were found to be susceptible. The lesions which we have produced in two species of monkeys are very similar, if not identical, to those which other observers have been able to induce in these animals with leprosy material direct from human subjects. But in addition we have also produced similar lesions in experimental monkeys with cultures of the organism.

In a later paper Soule and McKinley (415) reported that cultures of the ninth, tenth and eleventh generations of their organism isolated from leprous tissue when injected into older *M. rhesus* monkeys failed to produce lesions. Not only were there no nodular lesions produced but there was no evidence of any unusual local reaction following inoculation. By this time our culture of *Mycobacterium leprae* had been under artificial cultivation for more than a year and, as we have previously stated, the culture was if anything decreasing in volume rather than increasing. We have had a feeling that the suggestive lesions in monkeys first described by us were related to the age of the animals but we have no controlled experiment to establish this point. Also at this time we inoculated guinea pigs with 1 cc. of the suspension of our organism in the region of the inguinal lymph nodes. Eight weeks following the inoculations the animals appeared normal and upon autopsy the inguinal glands showed no lesions and no acid-fast organisms were found. Furthermore, we inoculated a number of mice (including white, dilute brown and several varieties of waltzers) and no lesions were produced. At autopsy six weeks later no organisms were demonstrated and no tissue changes were present which would lead one to suspect the existence of any leprous process in these animals.

As a result of these various experiments described by Soule and the author the following conclusions, which we felt were very conservative, were submitted:

The experiments described include (1) the experimental production of granulomatous lesions suggestive of early lesions of leprosy in two species of monkeys by intradermal inoculation of human leprosy material; (2) the cultivation of acid-fast (presumably *B. leprae*) bacilli from human leprosy nodules on several artificial mediums in various gaseous environments and (3) the experimental production of granulomatous lesions, suggestive of early leprosy, in two species of monkeys by the intradermal inoculation of cultures of acid-fast bacilli from human leprosy material grown on artificial mediums. We believe that the experiments indicate a step forward in the fulfillment of Koch's postulates for the causative agent in the disease of leprosy.

More recently McKinley and Verder (501) have been attempting a variety of methods in experiments designed to produce more progressive lesions in laboratory animals with leprosy material (tissue and cultures). It will be recalled that in the early monkey experiments of McKinley and Soule (5) the suggestive lesions which were produced healed spontaneously. It must also be borne in mind that similar lesions to these we have described may be produced with killed (heated) germs of leprosy taken from leprosy tissue and that not too much significance can be attached to the suggestive lesions in monkeys which we described. The problem in leprosy is, of course, to produce characteristic lesions in animals which will be progressive just as they are slowly progressive in man. Still, as we have previously pointed out in this review, no one can surmise what leprosy in an experimental animal should really be. It may be impossible to reproduce in any experimental animal the exact counterpart of leprosy as we have known it in man. On the other hand if it were possible to produce in animals *progressive* lesions which would not tend to heal spontaneously we would at least have something more than we have at the present time, and we believe that investigators in this field of work would be more likely to view such lesions with much more seriousness than the temporary lesions which have been produced so far. With this thought in mind the author, with his associate Dr. Verder, has attempted a somewhat new approach to the problem. While our work is not complete at the time this review is being written, still mention at least may be made of the progressive and persistent ulcerative lesions we have been able to produce in guinea pigs and monkeys under certain conditions. Knowing something of the chemistry of the acid-fast group it occurred to us that it might facilitate infection in experimental animals if we supplied the animal's tissues with an abundance of lipoid material along the lines suggested by Nègre (502) in his work on tuberculosis. We therefore extracted with acetone and water mixture lipoid material from the various tissues of the guinea

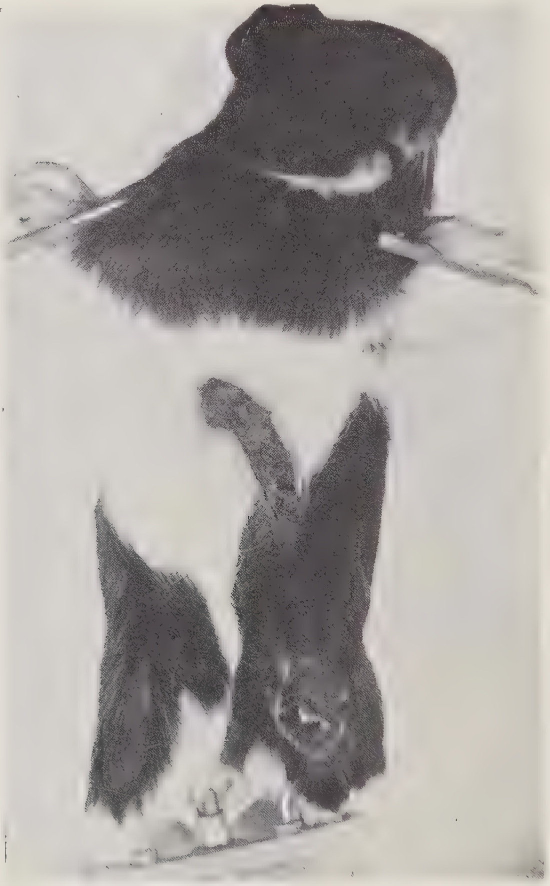

FIG. 10. ULCERATING LESIONS PRODUCED IN GUINEA PIG WITH MYCOBACTERIUM LEPRAE FOLLOWING PREPARATION OF THE ANIMAL BY INJECTION WITH LIPOID
(McKinley and Verder)

pig. After carefully distilling off the acetone these lipoids were sterilized in the autoclave. Guinea pigs were then injected subcutaneously in one groin with doses of 5 cc. of this lipoid suspension and *Mycobacterium leprae* was introduced subcutaneously in the opposite groin.

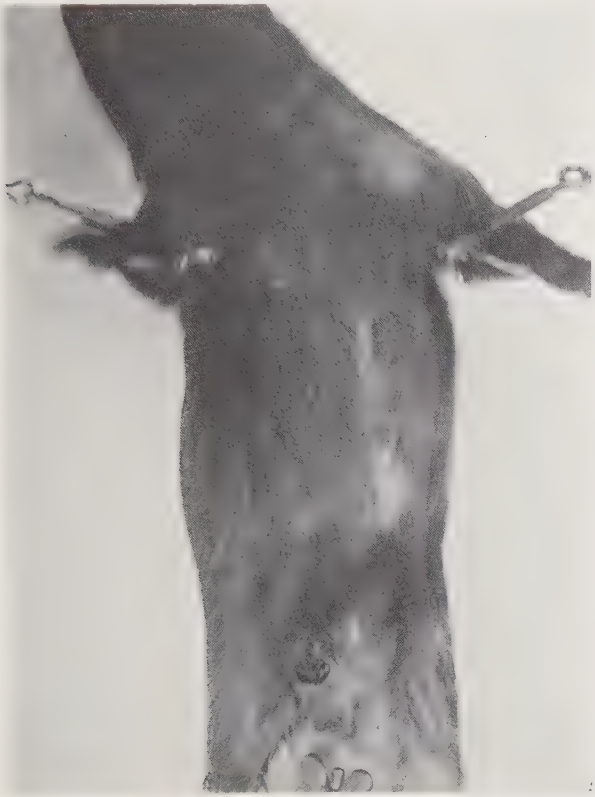

FIG. 11. ULCERATING LESION PRODUCED IN GUINEA PIG WITH MYCOBACTERIUM LEPRAE FOLLOWING PREPARATION OF THE ANIMAL WITH LIPOID
(McKinley and Verder)

On subsequent treatments the animals received lipoid in the other groin and *Mycobacterium leprae* again in the opposite groin. This method of treatment has resulted in some very marked ulcerative lesions in the guinea pig in which one may find acid-fast bacilli and these lesions in some instances have been very progressive. Further report of this work, however, cannot be made at this time since these studies are only now in progress. Similarly injections of glycerol have been employed in monkeys and vitamine deficient diets have been used in monkeys, guinea pigs and rats to lower resistance to the infection. Reports will appear later elsewhere.

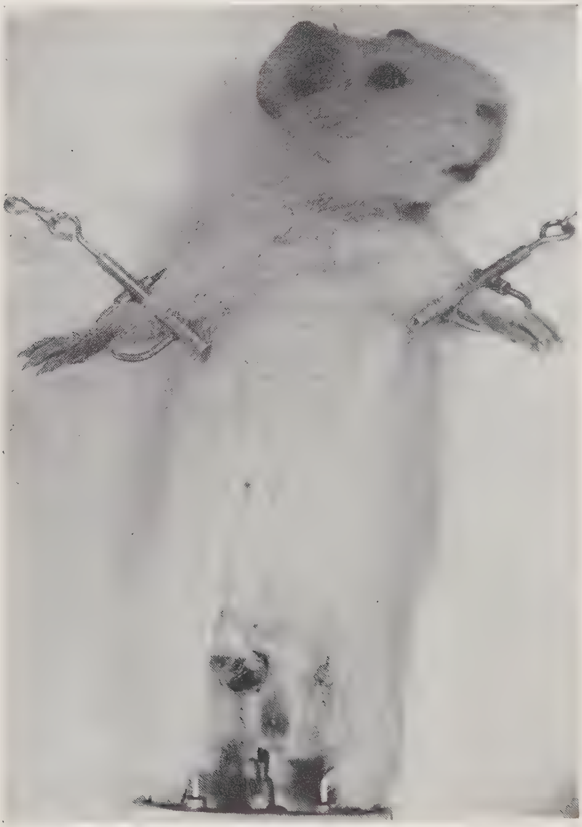

Fig. 12. Ulcerating Lesion Produced in Guinea Pig with Mycobacterium Leprae Following Preparation of the Animal with Lipoid
(McKinley and Verder)

In summing up the work to date on the cultivation of *Mycobacterium leprae* and the production of experimental lesions of leprosy in laboratory animals we feel constrained to lean far over on the conservative side of both questions. The author, in his work with both Soule and Verder, feels that in the work reported by this group we have perhaps the most promising advances in these two lines of investigation. We feel there is much evidence to support the view that we have actually cultivated *Mycobacterium leprae.* We feel that we have produced lesions in laboratory animals which are most encouraging to say the least. We realize, however, that Koch's postulates have not been definitely and positively fulfilled for *Mycobacterium leprae* and our efforts in the future will be to bring further experimental data to bear upon this problem. Meanwhile we are hopeful that other investigators elsewhere will attempt seriously and carefully to confirm our results. The controversial nature of this question has already been pointed out early in this review but we feel that the time has come when investigators should lay aside bias and prejudice concerning this question and make every effort to check honestly and rigidly any line of investigation which offers any encouraging hope whatever for the solution of this problem. Hasty studies and careless conclusions whether concerning our work or the work of other investigators are not in the best interest of the scientific advancement of this subject and are to be deplored. It is quite probable that many investigators in the past have been at least partly right in the conclusions they have drawn from their work and perhaps we too are only partly right in our study of this problem, but it usually requires the contributions of many investigators to establish fully the essence of proof of a complicated problem such as the etiology of leprosy.

Chemistry of "Mycobacterium leprae"

We have left a brief consideration of the problem of the chemistry of the germ of leprosy to the end of this review because we felt it was essential to present the background of the work on the cultivation of *Mycobacterium leprae* in order to interpret the work which has been reported on the chemistry of the organism. Manifestly the chemist has had only two types of material to work with in so far as the germ of leprosy is concerned. He has had his option of working with various strains of so-called *Mycobacterium leprae* or material collected from leprosy tissue itself which is, of course, abundant in so far as acid-fast bacilli are concerned, particularly in the nodular type of the disease. If he has worked with cultures of *supposed Mycobacterium leprae* then the question might properly be asked now whether he has actually worked with the true germ of leprosy. Chemical work then which has been reported based on present existing cultures may be regarded only as chemical studies on certain acid-fast bacteria and not necessarily on the germ of leprosy at all. In other words, as we have seen in this review, there is no proof that the many organisms claimed to be *Mycobacterium leprae* are actually the germs of leprosy. It is probable that most of them are not.

Of course it has been known for decades that the acid-fast organisms contain a waxy or fatty substance not common to other species of bacteria. There have been many papers dealing with this subject and the studies on the staining of *Mycobacterium leprae* are voluminous. The early work on nastin, a substance which was obtained from organisms isolated from lepers, will be recalled at this point. However, no very serious work was accomplished on the chemistry of the acid-fast group until the last two decades, and more particularly during the past ten years. While it will not be possible to review in this paper all of the work which has been done on the subject of the chemistry of acid-fast organisms, or more particularly so-called *Mycobacterium leprae*, reference will be made to a few of these studies since 1923.

The introduction of the oils in the treatment of leprosy at once raised the question as to how these agents acted. In 1923 Rogers (503) published a note on "defatting" the organism of leprosy by the injection of chaulmoograte and morrhuate. Paldrock (504) (505) was continuing his interest in the staining of *Mycobacterium leprae* and in 1926 studied organisms treated with ether by various staining methods, all of which emphasized the fatty substance contained in this organism. These papers were followed by several others by Paldrock (506) (507) (508) in 1927 in which he stated that he studied many organisms of so-called *Mycobacterium leprae* and that all of them invariably behaved identically on microchemical analysis. These several strains of so-called *Mycobacterium leprae* all contained free nucleic acid, bound nucleic acid as nuclear protein, karyonic acid in the granules, free lipoid in the membranes and also in the granules and lipoproteins in the granules. Chemically, Paldrock found all of these strains the same. In 1929 Schlossmann (509) published a brief study of the antigenic lipoids of several strains of *Mycobacterium leprae* and *M. tuberculosis.* The following year Markianos (510) stated that defatted antigen does not prevent rat leprosy wholly, but tends to retard, in a measure, the development of the disease. In a second paper he stated that the defatted antigen of rat leprosy acts favorably in the treatment of both rat and human leprosy. In 1923 Wells, De Witt and Long (511) had reviewed the chemistry of the tubercle bacillus and *Mycobacterium leprae* and in another review published the same year Long and Campbell (512) stated that in a comparative study of the lipids of acid-fast bacteria they found that the leprosy bacillus gave 9.7 per cent of total lipids. The lipid had a saponification number of 188 and contained 27.2 per cent of unsaponifiable matter.

In 1932 Emerson, Anderson and Leake (513) reported that in rat leprosy they found the tissues significantly lower in lipolytic activity than other tissues in infected or normal rats. They state that leprosy tissue from different animals is remarkably constant in lipolytic activity in comparison with other tissues in infected or normal rats. The work of Adams and his colleagues is well known. In 1932 Stanley and Adams (514) studied the surface tension of 120 acids and the same year Stanley, Coleman, Greer, Sachs and Adams (515) reported on the bactericidal action of certain synthetic organic acids towards *Mycobacterium leprae* and other acid-fast organisms. They employed seven strains of so-called *Mycobacterium leprae* obtained from the American Type Culture Collection and one unknown strain. They concluded that when a certain combination of physical properties is present in the molecules of these aliphatic acids, bactericidal action towards *Mycobacterium leprae* and other acid-fasts appears. One factor is the molecular weight with maximum action appearing ordinarily in molecules of 15 to 18 carbon atoms. Ability to form soapy solutions in aqueous solutions of sodium salts also seems important in effective acids. Also effective acids seem to be good surface tension depressants. Again, in 1932 Uyei and Anderson (516) and Anderson and Uyei (517) described their work on the chemistry of a strain of acid-fast organism which was isolated from a case of leprosy in Honolulu in 1909. This is known as Hygienic Laboratory Strain No.

370. The chemical analysis of this organism (3000 cultures) showed phosphatide 100.5 grams, acetone-soluble fat 289.5 grams, chloroform-soluble wax 444.8 grams, total lipoids 834.6 grams, polysaccharide 41.2 grams, dry bacillary residue 3389.8 grams and dry bacterial matter per culture 1.488 grams. They state that the figure for lipoids represents only those portions that can be extracted by alcohol-ether and by chloroform at room temperature. In their second paper these authors state that the phosphatide isolated from *Bacillus leprae* has been analyzed and found to be similar in composition to the phosphatides isolated from other acid-fast bacteria. They point out, however, that there are some differences. For example the phosphatide is exceedingly stable and cannot be hydrolyzed with dilute aqueous acid; the solid saturated fatty acid is not homogeneous and consists of palmitic acid with a slight admixture of a new fatty acid with high molecular weight which they could not identify. Also the authors state that two unsaturated fatty acids are present which on catalytic reduction are converted into palmitic acid and stearic acid. There was also a small amount of wax-like substance in the ether-soluble constituents. Anderson and Uyei state that when the phosphatide is saponified with dilute alcoholic potassium hydroxide, only the fatty acids and glycerophosphoric acid are split off, while the polysaccharide complex is left intact. This polysaccharide when hydrolyzed yields about two parts of mannose, one part of inosite and one part of a reducing hexose which is probably invert sugar or fructose. Some further studies on the polysaccharide have been reported recently by Newman and Anderson (518).

In 1932 Reed and Gardiner (519) studied the S and R types of a strain of so-called *Mycobacterium leprae* and concluded that they may be differentiated by acid agglutination. Also electrophoretic potential determinations indicated a similar type difference. They state that the iso-electric point of the S type is at pH 1.2 and the R type at pH 2.2. Acid agglutination occurred at about the same electrophoretic potential, namely at about 18.2 millivolts. These authors employed what they termed "well-established strains of S and R *Mycobacterium leprae*" grown on either solid media or Proskauer and Beck's fluid.

While, as we have pointed out, there are difficulties in attempting chemical studies of *Mycobacterium leprae* fundamentally because there is no accepted strain of the true bacillus of leprosy as yet, there is still another difficulty and one which may unfortunately cause the chemist further embarassment. Recently we have been hearing more about possible variants in the acid-fast group. Since dissociation as a phenomenon has been so well established with other bacterial species it is not surprising that investigators have looked for evidences of this phenomenon among the acid-fasts. Furthermore, it would not be surprising if variants were found in this group of microbes as they are found apparently in most other bacterial forms. In 1929 Zolkevitsch (520) reported that with radium emanations she was able to produce bizarre forms from Kedrowski's strain of so-called *Mycobacterium leprae.* Enormous threads and branched forms were quite commonly produced by this method. Later Kahn and Schwarzkopf (521) reported variants in cultures of *M. tuberculosis* and the bacillus of rat leprosy. These investigators employed animal passage, rapid transfers on glycerol broth containing normal inactivated rabbit serum and aging of cultures in the incubator as means of producing changes. They state that the leprosy strain 368 with which they worked mutates spontaneously in each direction to R and S on plates of Petroff's egg medium. In this question of variation with the acid-fast group of organisms we find then new complications not only for the bacteriologist, but also for the chemist as well. It still remains true, however, that given with certainty the true germ of leprosy and a reliable method of cultivating this organism *in vitro* we would be well along the way to success in the study of some of these other most fundamental problems.

Before taking up the summary of this review we should like to mention briefly one or two other recent contributions which seem to have in them the elements of some new thinking regarding the problem of leprosy and its etiology. Chiyuto (522) has recently described his observations on 40 children of leprous parents and states that a minute papulo-vesicular eruption, rather "goose-flesh" in aspect, was found in 16 cases, or 40 per cent. He states such lesions are leprotic in nature as confirmed histologically. Chiyuto states that the sites of the depigmented macules in children coincide with the regions that have been in prolonged skin to skin contact with leprous parents and confirm the fact that leprosy is contracted in early infancy and becomes clinically frank in adult life. He therefore feels that epidemiological studies on leprosy are valueless unless they include studies on infants and young children in the particular community under study. Also from the Philippines comes another recent paper by Rodriguez, Mabalay and Tolentino (523) concerning Gram-positive organisms in leprosy lesions in which no acid-fast organisms are demonstrable. These authors believe that the organisms which they find Gram-positive but not acid-fast are *in fact* the organisms of leprosy. They state that these organisms are not merely degenerated forms, since they are numerous in many cases of so-called "closed" or "incipient" cases of leprosy which have not undergone treatment. In these papers one finds evidence of a definite method of spread of leprosy by contact and also a suggestion why in some cases of leprosy it may be impossible to demonstrate the acid-fast *Mycobacterium leprae* in the lesions. Both of these observations are worthy of consideration in the future study of this disease. In the report of Rodriguez, Mabalay and Tolentino concerning the presence of a Gram-positive bacillus in tissues from lepers one should not lose sight, however, of the many instances in which diphtheroids have been described from such sources. In the course of all of our work on cultivation we have isolated during the past year five such strains which grow readily on blood agar. These strains of diphtheroids were first noted in tissue culture in which our acid-fast organisms have been growing. It has not been easy to separate our acid-fast organisms from these but we are certain that the nodules originally employed for cultivation experiments from lepers contained both the true germ of leprosy and these diphtheroids and we regard the latter as mere contaminants finding their locus in the devitalized tissue of the leper and of no consequence in so far as the actual disease process in concerned. The diphtheroid is apparently an organism quite commonly associated with leprosy-infected tissue but should be regarded only as a secondary invader and of no importance to the bacteriology of this disease.

SUMMARY

In the preceding review concerning the etiology of leprosy we have attempted to present this subject in the setting and with the background of a comprehensive literature which has accumulated during the sixty years since Hansen first described the germ of leprosy in leprosy tissue. It is, of course, no easy task to select from the literature the most important contributions bearing upon such a subject, particularly when the subject has been one of such controversy, but we feel that in the bibliography appended we have missed few, if any, of what might be termed the more significant contributions to this subject.

In tracing the scientific history of this disease we have seen that many claims and counterclaims have been made for and against many of the organisms which have been isolated from lepers. This was true in 1874 and it is still apparently true in 1934. Too often with investigations in this problem of leprosy, which has been of such a controversial nature, deductions have not always rested upon facts alone but partly upon prejudice and bias, and, as in other fields, these have to a large extent colored the discussions. This is perhaps only a very human reaction to a very human problem and was to be expected. The writer feels, however, that this state of mind in the scientific aspects of the problem has not contributed much towards the solution of the problem of either the cultivation of Hansen's bacillus, or the production of experimental lesions in animals with cultures. In fact it must be stated today, sixty years after Hansen first saw *Mycobacterium leprae*, that *there exists no absolute proof as yet* that any investigator during all of these years has actually succeeded in cultivating *Mycobacterium leprae in vitro*.

We are well aware that there are those investigators who will not be willing to agree with this statement, probably feeling that the organisms cultivated by them from the tissues of lepers represent the true *Mycobacterium leprae*. We can appreciate this point of view. Yet the author with his colleagues, who have also advanced cultures which they feel are probably *Mycobacterium leprae*, are of the opinion that this is the only fair statement which can be made at this time in the matter of cultivation of the leprosy bacillus. We feel definitely that we have an organism which has more in its favor than any other organism which has been submitted as *Mycobacterium leprae*. We feel that we have perhaps gone somewhat further in establishing this organism as *Mycobacterium leprae* through animal experimentation.

Yet the organism we isolate from leprosy tissue is grown with only great difficulty and is very sparse in its growth, and we have not succeeded in producing in laboratory animals the counterpart of leprosy in man. In keeping with the controversial nature of much of the work on the bacteriology of leprosy which has characterized the past are the recent papers of Duval and Holt (524) and Holt (525) who, in rather hasty studies of the methods, and certainly with lack of much experience with them, have attacked the work of Soule and Verder with the author. The author and his colleagues feel that the bacteriology of leprosy is a scientific problem to be seriously studied and does not constitute an arena for controversy just for the sake of controversy. With this we have no interest. On the other hand it is obvious that repeated repetition of work and methods is most essential in this problem, but we have learned after several years of experiments with the gaseous tension method and with tissue culture that there are many pitfalls for the uninitiated. Furthermore, we recognize that these methods need to be further perfected in order to obtain even better results and this we are attempting to do. Meanwhile, no doubt, other investigators will be critical of the rather feeble results which we are able to obtain in the cultivation of this organism but we hope that at least serious effort will be made to confirm our findings up to this point. Anything less than this is no contribution to the subject. It cannot be done within a few short weeks.

As for animal experimentation, we feel again that the only fair statement which can be made at the present time is that no investigator has to-date succeeded in producing the counterpart of human leprosy in any experimental animal. Naturally we include our own attempts in this direction in this statement. It is hopeful, however, that new approaches to this problem may eventually lead to accepted progressive lesions of the disease in lower animals. If this can be accomplished in a satisfactory manner then there is also the hope of eventually establishing beyond peradventure of a doubt the validity of a suspected culture of *Mycobacterium leprae* as the actual etiologic agent of leprosy.

Regarding the chemistry of *Mycobacterium leprae* it must again be pointed out that, until an authentic culture of *Mycobacterium leprae* can be obtained in the test tube, it is useless to pretend that we know anything definite regarding the chemistry of the true germ of leprosy except by analogy with what is known concerning other bacteria in the acid-fast group of organisms. It is quite probable that all the members of the acid-fast group have many chemical characteristics in common but the chemical study of *Mycobacterium leprae* up to the present time cannot be regarded as enjoying the same status as *M. tuberculosis* in this regard.

What then is the etiology of leprosy? Investigators the world over still believe that Hansen's bacillus is the cause of leprosy. There seems to be no good reason to question this organism which is so constantly associated with lesions of this disease. The final cultivation of this organism on artificial media will one day be accepted and another chapter in the study of this disease will be brought to a close.

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